U.S. DEPARTMENT OF COMMERCE
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PB-289 789

Assessment of Health Effects of Benzene Germane to Low-Level Exposure

(U.S.) Environmental Protection Agency, Washington, DC

Sep 78

United States
Environmental Protection
Agency

Office of Health
and Ecological Effects
Washington DC 20460

EPA-600/1-78-061
September 1978

Research and Development

PB 289789

EPA

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TECHNICAL REPORT DATA
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1. REPORT NO. EPA 600/1-78-061	2.	3. RECIPIENT'S ACCESSION NO. PB289789
4. TITLE AND SUBTITLE ASSESSMENT OF HEALTH EFFECTS OF BENZENE GERMANE TO LOW LEVEL EXPOSURE		5. REPORT DATE September 1978
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Office of Health and Ecological Effects Office of Research and Development U.S. Environmental Protection Agency Washington, D.C. 20460 (and various contractors)		10. PROGRAM ELEMENT NO. 1HA630
		11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Office of Research and Development U.S. Environmental Protection Agency Washington, D.C. 20460		13. TYPE OF REPORT AND PERIOD COVERED Final
		14. SPONSORING AGENCY CODE EPA/600/18

15. SUPPLEMENTARY NOTES

16. ABSTRACT

This report on the health effects of benzene assesses the teratagenicity, mutagenicity, carcinogenicity, and hematopoietic effects from benzene germane to low level exposure.

Epidemiological data strongly indicate that benzene is a human leukemogen although no animal models have been developed.

This is one of three documents which will be used by EPA's Office of Air and Waste Management, and by the Administrator, to determine what actions, if any, should be taken against benzene under the Clean Air Act.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Dose-Response Health Effects Toxicology Air Pollution Exposure Carcinogen	Environmental Pollution Benzene Leukemia Blood Dyscrasias	

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 119
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE PCA06/MF A01

EPA Form 2220-1 (9-73)

EPA-600/1-78-061

September 1978

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U.S ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Ecological Effects
Washington, D.C. 20460

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PREFACE

The Environmental Protection Agency has prepared three documents concerning the health effects of benzene on the general population:

1. A health effects assessment
2. An environmental exposure assessment
3. A population risk assessment, based on the data presented in the first two documents.

This report is the health effects assessment; it will be used by the Environmental Protection Agency's Office of Air and Waste Management and by the Administrator to determine the scientific basis for possible actions regarding benzene under the Clean Air Act.

Earlier drafts of this report have been reviewed by many individuals and organizations. On January 18, 1978, the Environmental Health Advisory Committee of the Environmental Protection Agency's Science Advisory Board held a special "readers' meeting" concerning the report. Members of the Environmental Health Advisory Committee who served as special readers are:

Dr. James H. Sterner (chairman), College of Medicine, University of California (Irvine);

Dr. Samuel S. Epstein, School of Public Health, University of Illinois (Chicago);

Dr. Bernard D. Challenor, College of Physicians and Surgeons, Columbia University;

Dr. Jenifer L. Kelsey, Yale University School of Medicine.

Drafts of the three benzene assessment reports were reviewed by the Environmental Health Advisory Committee in public session on February 3, 1978. The members of EHAC, in addition to those listed above, are:

Dr. Norton Nelson (chairman), New York University Medical Center;

Dr. William J. Darby, The Nutrition Foundation, Inc.;

Ms. Dorothy B. Hood, Haskell Laboratory for Toxicology and Industrial Medicine;

Dr. Bailus Walker, Jr., Department of Environmental Services, Government of the District of Columbia;

Dr. James L. Wittenberger, School of Public Health, Harvard University;

Dr. Gerald N. Wogan, Department of Nutrition and Food Science, Massachusetts Institute of Technology.

Drafts of this report were also reviewed at an interagency meeting on December 13, 1977. Representatives of other Executive Branch departments attending the meeting included:

G.W. Siebart, representing the Department of Defense, Office of the Secretary of Defense;

Joan Cloonan, representing the Department of Justice;

Gene Lehr, representing the Department of Transporation;

Steven Bayard, representing the Consumer Product Safety Commission, Office of Engineering Sciences and Health Sciences;

Dorothy Canter, representing the Consumer Product Safety Commission, Office of Engineering Sciences and Health Sciences; and

B. Osheroff, representing the Department of Health, Education, and Welfare.

Review copies of this document have also been provided to other government agencies and to industrial and public interest groups as the result of a notice that appeared in the Federal Register November 9, 1977, on page 58440.

All comments and criticisms received at these meetings and in response to the Federal Register notice have been reviewed and incorporated into the document as deemed appropriate.

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ACKNOWLEDGMENT

This document was prepared by EPA's Office of Research and Development with extensive help from a team of consultants led by Bernard D. Goldstein, M.D. Major contributions were by Carroll A. Snyder, Ph.D., Robert Snyder, Ph.D., and Sandra R. Wolman, M.D. The views represented in this document are those of the EPA and not necessarily those of the consultants.

SUMMARY AND CONCLUSIONS

This report presents the research findings on benzene toxicity that are relevant to assessing human health risks at environmental exposure levels. Following are the principal conclusions to be drawn from this report.

1. Benzene exposure by inhalation and other exposure routes is strongly implicated in three pathological conditions that may be of public health concern at environmental exposure levels:

   - leukemia, especially acute myelogenous leukemia
   - pancytopenia (including aplastic anemia)
   - chromosomal aberrations.

2. Given the epidemiological data obtained in occupational exposure studies, one can argue convincingly that benzene is a human leukemogen. The exposure data in these studies do not allow a scientific derivation of a dose-response curve. Most studies in which exposure levels were determined involved doses in the range of 100 to 500 ppm, though in some the benzene concentrations were lower.

3. Since most studies concern middle-aged to elderly males who were occupationally exposed to benzene, no conclusions can be drawn about differences in susceptibility of other populations.

4. Currently there is no convincing evidence that benzene causes neoplasias, including leukemia, in animals. Failure to induce leukemia in animals could be due to an as-yet-unknown cocarcinogen required to evoke the leukemogenic response initiated by benzene.

5. Hematotoxicity, particularly pancytopenia, has been observed in both humans and animals following exposure to benzene. The toxicity does not follow exposure to other compounds such as toluene and xylene commonly associated with benzene environmentally.

6. Humans who develop hematologic abnormalities due to benzene exposure have a greatly increased probability of developing leukemia and aplastic anemia, a finding consistent with the thesis that benzene is leukemogenic.

7. Long-term occupational exposures of workers to benzene at levels as low as 20 ppm, but generally at levels greater than 100 ppm, have resulted in various signs of hematotoxicity.

8. Two effects, as yet unconfirmed, of potential significance have been reported at occupational exposure levels of 3 to 15 ppm. The effects are 1) an increase in red blood cell levels of deltaaminolevulinic acid, a precursor in the heme biosynthetic pathway, and 2) a decrease in the mean serum complement of the blood.

9. Available data from studies in which measurements ranged from 25 to 150 ppm strongly suggest that chromosome breakage and rearrangement can result from chronic exposure to benzene; in at least one study, significant effects were noted at 2 to 3 ppm (time-weighted average). These aberrations have been observed to persist in lymphoid and hematopoietic cells after removal from benzene exposure. Since a favored mechanism for leukemia development is somatic mutation, the persistence of chromosomal aberrations, coupled with clinical observations of chromosomal abnormalities in human leukemic cells, support the thesis that benzene is a leukemogen. A dose-response relationship has not been demonstrated for benzene-induced chromosome aberrations. This lack may result from variations in individual susceptibility.

10. Benzene toxicity probably occurs via a toxic metabolite.

11. In animals, benzene accumulates in lipoid tissue such as fat and bone marrow, and benzene metabolites concentrate in the liver and bone marrow. The concentration of metabolites in the bone marrow exceeds that in the blood.

12. The accumulation of benzene metabolites in bone marrow along with the coincidental covalent bonding of benzene to solid residues of bone marrow is consistent with a phenomenon of toxico- and carcinogenesis shared by many other chemicals.

SECTION 1
INTRODUCTION

There is substantial evidence that concentrations of benzene encountered in the work place (in the United States and elsewhere) have caused diseases of the blood and bone marrow in general (e.g., blood dyscrasia, pancytopenia) and leukemia in particular (especially acute myelogenous leukemia). Because current policy of the Environmental Protection Agency (EPA) states that there is no zero risk level for carcinogens, benzene has been listed by EPA under Section 112 of the Clean Air Act as a hazardous air pollutant.

This report is an assessment of the health effects of benzene germane to low-level exposure; it is largely a review and evaluation of the scientific literature relevant to determining the human health effects of environmental exposures to benzene. Most of what is known concerning the effects of benzene on human health has been learned by studies of persons exposed to benzene in the workplace. Virtually no information is available that describes the health effects of nonoccupational exposures of the general populace to benzene. Our evaluation of potential environmental health effects, then, must be based upon what we know of the mechanisms of benzene toxicity and its genetic implications and of the effects of benzene on animals and on human beings. This report is structured accordingly.

Section 2 introduces some of the major biomedical concepts that are pertinent to assessment of the health effects of benzene. Following a brief discussion of benzene metabolism in animals and in humans, the cytogenetic effects of benzene are considered, particularly its effects on chromosomes.

The major portion of the report deals with assessments of benzene toxicity in animals (Section 3) and in man (Section 4). These latter analyses focus on two forms of benzene-induced disorders: 1) pancytopenia, defined as the diminution of all formed elements in the blood, and 2) leukemia, defined as a proliferation and accumulation of mature and immature white blood cells (leukocytes) in blood and/or in bone marrow, leading to the impairment of normal function.

SECTION 2

BENZENE METABOLISM; CYTOGENETIC, AND EMBRYONIC EFFECTS

BENZENE METABOLISM

Metabolism in Animals

Most of the benzene that enters the body is excreted via the lungs in exhaled air.[5,46] Study of the distribution of benzene and its metabolites in animal organs shows that free benzene accumulates in lipoid tissue such as fat and bone marrow. High concentrations of benzene metabolites can be observed in liver tissue and in bone marrow. It is particularly significant that the concentration of metabolites in bone marrow exceeds that in blood.[5] Repetitive administration of benzene leads to accumulation of both benzene and its metabolites in these organs and to covalent binding of benzene metabolites to liver and to solid residues in bone marrow.[60]

The metabolic pathway of benzene in liver is shown in Figure 1.[61] The initial step appears to be a reaction mediated by the mixed-function oxidase.[21] This enzyme is inducible, so that pretreatment with benzene, phenobarbital, or 3-methylcholanthrene can increase the rate of benzene metabolism.[21,56,59] The direct product of the interaction of benzene with mixed-function oxidase is probably an arene oxide that is highly reactive. It can spontaneously rearrange to form phenol, undergo enzymatic hydration followed by dehydrogenation to form catechol or a glutathione derivative (phenylmercapturic acid), or bind covalently with cellular macromolecules. Formation of hydroquinone or of trihydroxylated compounds probably is the result of several reactions with hydroxylating enzymes.

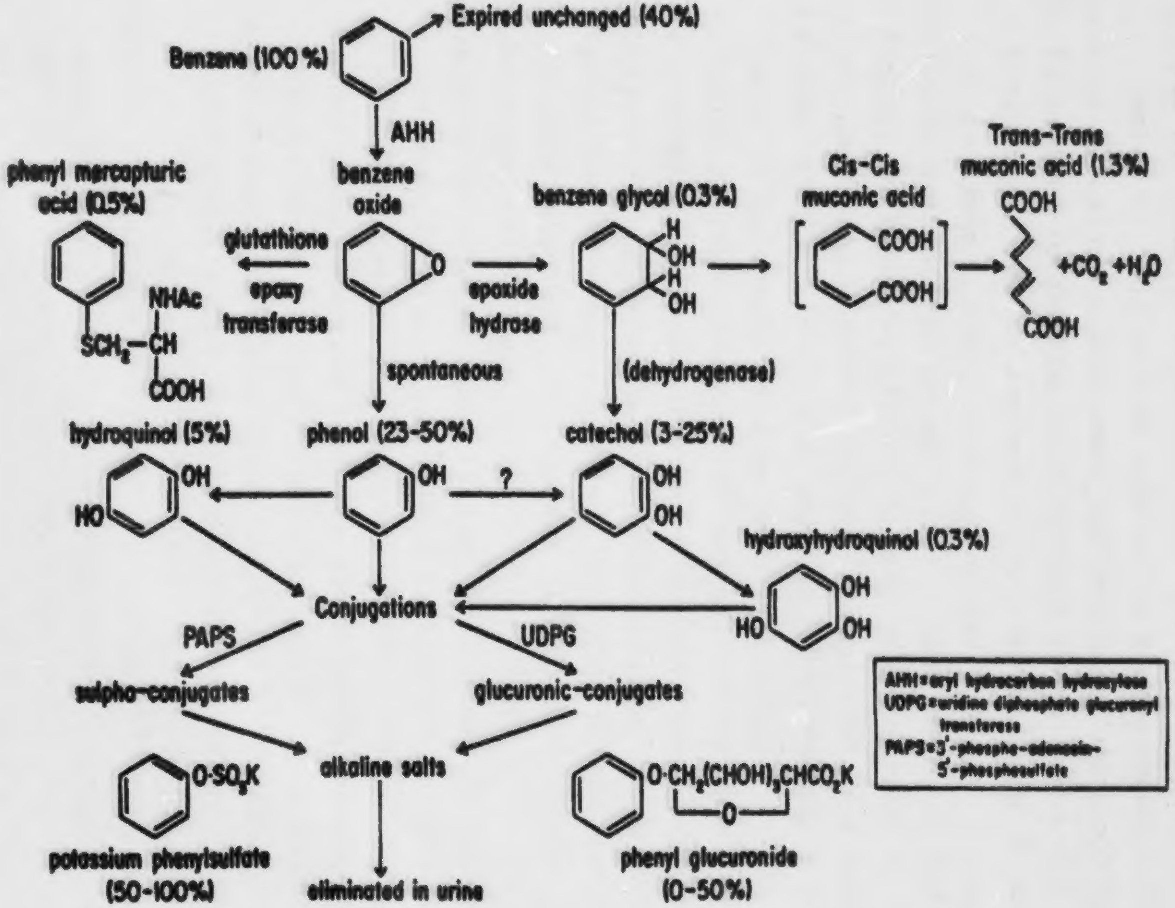
Benzene (100 %)
Expired unchanged (40%)
AHH
benzene oxide
phenyl mercapturic acid (0.5%)
glutathione
epoxy transferase
NHAc
SCH2—CH
COOH
epoxide hydrase
benzene glycol (0.3%)
H
OH
H
OH
Cis-Cis muconic acid
COOH
COOH
Trans-Trans muconic acid (1.3%)
COOH
COOH
+CO2 +H2O
spontaneous
(dehydrogenase)
hydroquinol (5%)
phenol (23-50%)
catechol (3-25%)
OH
HO
OH
?
OH
OH
hydroxyhydroquinol (0.3%)
OH
HO
OH
Conjugations
PAPS
UDPG
sulpho-conjugates
glucuronic-conjugates
AHH = aryl hydrocarbon hydroxylase
UDPG = uridine diphosphate glucuronyl transferase
PAPS = 3'-phospho-adenosin-5'-phosphosulfate
alkaline salts
O·SO3K
O·CH2(CHOH)3CHCO2K
O
potassium phenylsulfate (50-100%)
eliminated in urine
phenyl glucuronide (0-50%)

Figure 1. Metabolic pathway of benzene in liver.

Benzene Metabolism in Man

Most metabolic studies of benzene in man have been concerned either with uptake and excretion of unchanged benzene via the breath or with measurements of benzene metabolites in urine. Nomiyama and Nomiyama[44] exposed volunteers to a series of solvent vapors and found that among six subjects exposed to benzene at 52 to 62 ppm for 4 hours, retention of benzene in the respiratory system decreased and then became constant after 3 hours at 30.2 percent of the inspired dose. There was no distinction attributable to sex of the subjects. Excretion, as measured in exhaled air after removing the subject from the benzene-laden atmosphere, was about 16.8 percent. Net uptake, i.e., the sum of uptake and excretion, was 46.9 percent. These authors[43] went on to show that when the logarithm of the benzene concentration in expired air was plotted against time, the excretion pattern described a hyperbole that could be expressed mathematically and that yielded three rate constants to describe the phenomenon. The subjects continued to excrete benzene in the exhaled air for as long as 15 hours. Hunter,[27] in studies of people exposed to 100 ppm benzene, detected benzene in the expired air 24 hours later. He suggested that measurements of benzene in expired air could be used to estimate benzene content of the inspired air by extrapolation.

Phenol content of the urine is often measured after benzene exposure. Maximum concentrations are thought to occur within 2 hours after exposure.[27] The major conjugated form appears to be ethereal sulfate until phenol levels of the urine reach 400 mg/liter, at which point glucuronide begins to appear.[58]

Teisinger et al,[64] who exposed humans to benzene at 100 ppm for 5 hours, reported that 46 percent of the dose was retained. Of that amount, 61 percent was recovered as phenol, 6.3 percent as catechol, and 2.4 percent as hydroquinone. In these studies the major monohydroxylated metabolite and the two major dihydroxylated metabolites observed by Parke and Williams[46] in rabbits were also observed in man.

Relationship of Benzene Metabolism to Benzene Toxicity

Since Parke and Williams[46] suggested in 1954 that a metabolite of benzene is responsible for benzene toxicity, evidence to support that hypothesis has mounted. Nomiyama[42] demonstrated that inhibition of benzene metabolism protected rats against benzene-induced leukopenia. Andrews[5] reported that when benzene metabolism was inhibited with toluene the subjects were protected against benzene-induced reduction of red cell production. Animals have been protected against benzene toxicity when pretreated with phenobarbital,[13,28] probably because phenobarbital stimulates benzene metabolism in liver, which leads to detoxification and thereby reduces the amount of benzene available for formation of the toxic agent in bone marrow. The specific metabolite that produces benzene toxicity has not yet been identified, but likely candidates are benzene oxide, catechol, and hydroquinone, or the corresponding semiquinones.

The demonstration that reduction of red cell count during benzene treatment is accompanied by accumulation of benzene metabolites in marrow and coincidental covalent binding of benzene to solid residues of marrow[60] suggests a phenomenon in toxico- and carcinogenesis shared by a variety of other chemicals, such as acetaminophen,[30] bromobenzene,[70] hydrazine derivatives,[38] parathion,[41] and many others.[29]

Although further studies are required to prove the hypothesis, it seems likely that benzene, like many other chemicals, exerts its toxicity by formation of a toxic metabolite.

CYTOGENETIC AND EMBRYONIC EFFECTS

Concepts

Mutagens and Carcinogens--

Benzene is believed to affect chromosomes, and chromosomal aberrations have been sought as indications of a biologic response to benzene for logical reasons. Somatic mutation has long been accepted as a critical event in the initiation or maintenance of malignant change, although the concept is not unchallenged. Focus on sites of genetic damage is based partly on observations of the prolonged delay from the time of exposure to a carcinogen until the advent of malignancy, such delay being consistent with perpetuation of the original damage in the genetic system. Further, many lines of evidence indicate that most, if not all, carcinogens are mutagens.

Rapid, convenient, accurate, and inexpensive systems for mutagen testing are available for evaluation of point mutations in prokaryotic cells;[4] nevertheless, the assessment of damage to mammalian chromosomes is probably more directly relevant to estimations of human health hazards from mutagens. If a cell shows sufficient chromosome alteration that further cell division is interrupted, then from a reproductive point of view that cell is dead and the damage is toxic. If, on the other hand, the chromosome alteration does not interfere with cell division and can be replicated, then it constitutes a mutation, a structural change in the genome that presumably alters cell function. Chromosomal breaks, which may be repaired, are not mutational events (in the sense of being heritable). However, each occurrence increases the probability of formation of a structural aberration and therefore of a mutation.

Investigations aimed at evaluating effects of benzene have appropriately concentrated on changes in cell nuclei, metabolism of deoxyribonucleic acid (DNA), cell division, and chromosome alterations. All these constitute direct measures of changes in quantity, structure, organization, or function of the cellular DNA. Moreover, some of these changes are heritable and imply permanent changes in the genome of the affected cell.

Clastogens and Mitotic Poisons--

The use of chromosome studies to monitor possible environmental mutagens should not be limited to evaluations of chromosome-breaking or "clastogenic" effects on cells arrested in metaphase. If the cells are analyzed without conventional pretreatment with mitosis-arresting agents or hypotonic solutions, abnormalities in the anaphase can be identified. These include multipolar mitoses, imperfect or unequal separation of chromosomes, and bridges interfering with reconstitution of the daughter nuclei. Some abnormalities may be detectable only in cells recovering from the effects of a chemical. During exposure the affected cells may be totally blocked from entering mitosis. Thus, in evaluating the potential action of a chemical as a chromosomal mutagen the investigator must look for both clastogenic and antimitotic effects. The latter may be especially important in chemicals that do not induce point mutations.

The effectiveness of benzene as a mitotic poison has been amply demonstrated. Decrease in DNA synthesis has occurred in cultured human cells[12,34] and in bone marrow of rats and rabbits after treatment in vivo.[9,33,37,39,62,63] The total numbers of nucleated cells, and, in some cases, the mitotic indices have declined. Inhibition of cell proliferation has been shown most often by decrease in uptake of radioactive-labelled thymidine, a DNA precursor. Although these may not be the most sensitive of indices, they are clearly and directly relevant to cell survival and reproductive fitness. Furthermore, both numerical and structural chromosome aberrations have been described that could be interpreted as either toxic or mutational damage. These include loss or gain of parts of chromosomes, whole chromosomes, or chromosome sets, in addition to exchanges that result in morphologically aberrant chromosomes.

Anaphase studies on human cells have not yet been reported but are under way in several laboratories. Morishima and his colleagues have described appropriate conditions for testing human material.[40]

Cytogenetic Aberrations in Leukemia--

The assumption that chromosomal mutation is etiologically important in the development of leukemia has been strengthened by observations of abnormalities in human leukemic cells. The close association of the Ph^1 translocation with chronic myelogenous leukemia is well-known,[45,54] and specific chromosomal abnormalities have been reported with other forms of leukemia.[55,57,67] These abnormalities appear to be specific to each disease entity, confined to the leukemic cells, and clonal (indicating a probable single-cell origin). Therefore, it is clearly important to investigate the actions of potential leukemogens with particular emphasis on their ability to cause site-specific chromosomal lesions. It is, however, even more likely that the initial damage caused by most carcinogens is nonspecific, causing a genetically more variable population of cells. This, in turn, increases the probability that an abnormal proliferative state will arise (or be selected).

Cytogenetic Studies of Animals

Studies of benzene effects have been conducted in many species, including rats, mice, rabbits, and newts. These studies have included whole-animal exposures and effects of benzene on cells *in vitro*; they have been based on either acute or chronic and repeated exposures. The results of such studies are difficult to evaluate since they differ not only in the biologic end-point chosen, but also in species, routes of administration, and dosage. Since few of the studies have involved inhalation exposure, their relevance to problems of human disease may be questioned.

Unpublished studies by Wolman et al evaluated chromosomal findings in rats chronically exposed to 300 or 100 ppm benzene. Within 10 weeks there was a striking and persistent increase in chromosome breaks and aneuploidy (deviation from the normal diploid chromosome number) in the bone marrow of treated (300 ppm) animals. The increase following 100 ppm exposure was not as great and not of clear statistical significance.

Increased chromosome breakage in several species has been reported. Rats exposed to benzene subcutaneously over a period of 12 days showed highly significant increases in chromosome aberrations of bone marrow cells over untreated and toluene-treated controls.[35] Classification of both gaps and breaks as aberrations complicates interpretation of these findings (since gap rates vary more with the interpreter and with preparation) and inflates the aberration rate. For example, although significant increases in aberrations were also found in the toluene-treated controls in this study, the benzene-treated group was the only one in which breaks were more common than gaps as aberrations. Exchange figures such as might result from abnormal repair after breakage (i.e., ring forms, translocations, and dicentrics) were rarely seen. Another, more acute exposure (2.0 ml benzene/kg body weight for 12 to 72 hours) in rats produced similar findings.[47] Increased numbers of chromatid breaks were found at almost every exposure interval, although the responses of individual animals varied considerably. Chronic exposure to injection of 0.2 mg/kg per day in rabbits (up to 18 weeks of treatment) also resulted in a high frequency of aberrations; since less than 15 percent of the aberrations reported were breaks, the significance of this study[33] is not established. Again, exchange figures, dicentrics, and hyperploid cells were rare. In each of these animal-exposure studies only a single dosage of benzene was used. Thus, although different exposure times in different species can induce increases in chromosome aberrations, there is no clear evidence for a dose-dependent response to benzene exposure. Furthermore, none of these studies presents data suggestive of mutational rather than toxic damage. Very few experiments have addressed the question of direct interference with benzene-induced abnormalities and possible therapeutic routes.

Studies of dividing erythroblasts taken from the amphibian newt (*Molge vulgaris L.*)[52,53] are of particular interest because of their demonstration of anaphase abnormalities. Young animals injected with water-saturated solutions of benzene were bled 6 to 12 hours later and a drop of tail blood was used for coverslip culture. At the time of sampling, 38 percent of mitoses were arrested in late metaphase. Another 28 percent showed evident anaphase abnormalities, of which 20 percent were migration arrests, 3 percent were anomalies of numerical distribution, and 3 percent were anaphase bridges. The remaining 2 percent showed small subgroups of chromosome condensations outside the two poles of the newly forming nuclei. Observations over several hours showed that these mitotic abnormalities resulted in unequal nuclear divisions, polynucleated cells, and atypical nuclei. Prophase and early metaphase anomalies were never found at the doses used in these studies (up to 54 mg of benzene per animal).

Cytogenetic Studies of Man

Experiments--

A few experimental observations have been made on the responses of cultured human cells to addition of benzene to the culture medium. Increased incidence of breaks and gaps was observed in leukocytes (white blood cells) and cancerous cells after brief exposure to 1.1 or 2.2 x 10^{-3} M benzene *in vitro*.[12,34]

At the higher dose a decrease in DNA synthesis interfered with clear correlation of dose to the incidence of breaks. These findings were considered to be toxic damage. Another experiment[23] in which peripheral blood lymphocytes stimulated by phytohemagglutinin (PHA) were exposed to benzene during 72-hour culture revealed both numerical and structural alterations in the treated cells. Aneuploidy was seven times more frequent in the treated populations than in controls, and chromosome breakage was seen in 11 percent of the treated cells as compared with 1 percent in the controls.

Chromosome Studies and Hematologic Disease--

In contrast to the paucity of experimental data, there is an abundance of reports on chromosome studies in exposed populations and of case reports on leukemia patients. The case reports are particularly difficult to evaluate and compare. Some individuals were exposed to benzene vapor above permissible levels,[10] but in many cases the exposure levels were unknown. The total periods of exposure ranged from brief and acute to as long as 22 years.[15] The times between exposure and the development of disease or death also varied greatly. Most of all, the endpoints of disease were not comparable. The various reports include diagnoses of acute intoxication,[10] death with massive bleeding and extramedullary hematopoiesis,[10] benzene leukemia,[15] acute myeloid luekemia,[25] acute erythroblastosis,[36] erythroleukemia,[16] acquired aplastic anemia,[11,49] acute lymphoblastic leukemia,[1] myelofibrosis, and chronic myelogenous leukemia.[69] Chromosome abnormalities have been present in industrial workers in association with hematologic pathology[23] or pancytopenia.[14]

Prior blood transfusions, the use of PHA-stimulated lymphocytes in some cases and bone marrow in others, and the lack of chromosome breakage rates in controls also hamper interpretation of the results of chromosome studies. Nevertheless, certain trends appear amid this mass of data. Additional chromosomes were identified in several cases,[1,10,15,50] and in two cases the additional chromosome was identified as a member of the C group. Both were cases of acute leukemia, in which additional C-group chromosomes (usually number 8) are frequently found;[55] therefore this does not, as one reviewer suggested,[24] constitute evidence of benzene etiology. Persistence of abnormal chromosomes long after exposure and illness was also reported.[51] Tetraploidy or polyploidy was found in several instances.[11,15,36,49] Increased chromosome breakage was reported but not well-documented.

It is important to emphasize that the end stage of exposure to benzene was as variable in alterations of the karotype, or chromosome "package," as it was in clinical manifestations. Indeed, the karyotypic changes may well have reflected the disease state more than they reflected the (presumed) inducing agents.

Occupational Exposures--

The clearest picture of the relationship between benzene exposure and chromosome changes emerges not from experimental studies but from studies of occupationally exposed workers. Tough and Court-Brown observed unstable* chromosome damage in cultured lymphocytes from workers exposed to benzene solvents.[65] They and their collaborators expanded the study[66] to include groups from three factories and sex-matched controls. The first group of 20 men had been exposed to benzene at factory A for periods of 1 to 20 years and were tested 2 or 3 years after exposure ended. The second group of 12 had worked periods of 6 to 25 years in areas where benzene was present (factory B), the exposure ending approximately 4 years prior to the study. The third group of 20 had worked for periods of 2 to 26 years in a closed distillation plant (factory C). In each instance controls were selected from nonexposed individuals in the same factories. Available measurements of atmospheric benzene were 25 to 150 ppm in factories A and B and approximately 12 ppm in factory C. The results indicated significant increases in unstable aberrations in exposed workers from factory A, in both exposed workers and controls in factory B, and in neither group in factory C. Furthermore, the exposed workers at factory A were older than their controls, and the authors demonstrated significant increases in aberrations in the general population with increasing age.

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* Unstable aberrations include open breaks, fragments, ring and multicentric chromosomes, and exchange figures. Stable aberrations include deletions, translocations, inversions, trisomies, and other alterations of chromosome number.

Several other investigators have reported increases in chromosome breakage or in stable and unstable aberrations in healthy workers.[19,26,31] In one report[26] the atmospheric concentrations of benzene were less than 25 ppm. More compelling results were obtained by Forni and co-workers,[17] who compared data on 34 workers in a rotogravure plant with those of matched controls. The group of workers was subdivided; 10 individuals had been exposed to benzene for periods of 1 to 22 years (measurements of benzene in the plant during a brief single period ranged from 125 to 532 ppm). These 10, with the remaining 24 workers, were then exposed to toluene for periods up to 14 years at levels ranging up to 824 ppm. The age- and sex-matched controls had no history of exposure to either solvent. The findings in workers exposed only to toluene were not significantly different from those in the controls, but the group that had been exposed to benzene showed increases in both stable and unstable chromosome aberrations ($p < 0.01$). Another study[18] of 25 subjects who had recovered from clinical "benzene hemopathy" indicated that increases in both types of aberrations persisted several years after cessation of exposure, although there was some decrease in the proportion of unstable aberrations. Average values of unstable abnormalities in the exposed group were 3 times greater than in the controls, and of stable chromosome abnormalities, 30 times greater. Researchers in a Swedish study[8] observed road-tanker petrol delivery drivers, crew members of petrol-carrying ship tankers, and employees at petrol filling stations to detect chromosome breakage and compared their findings with those observed in milk tanker delivery drivers and a group of industrial gas workers exposed occupationally to benzene. The results, though suggestive of a relationship between benzene (5 to 10 ppm) and chromosome damage, are not conclusive and are not helpful in demonstration of dose-related effects.

A very recent report[32] by Dow Chemical contains data indicating that occupational exposure of 52 workers to benzene for periods from 1 month to 26 years (average exposure time, 56.6 months) induced in peripheral lymphocytes chromosome breaks, dicentric chromosomes, translocations, and exchange figures at rates 2 to 3 times those found in controls (preemployment personnel). Although the figures were small, totalling 15 aberrations, the large number of cells examined (approximately 20,000) supports the significance of the differences ($p = 0.005$). A difference in the average age of the worker cohort (39.3 years) and of the controls (26.6) is not considered confounding since the difference is small; further, the cytogenetic effect most often associated with aging is chromosome loss rather than the chromosomal abnormalities reported. The authors, significantly, reported that the percentage of individuals with changes was larger in the group with the increased incidence of changes than in the unexposed control group. Thus the aberrations apparently were not confined to a few individuals in the exposed group. The authors estimate that the time-weighted 8-hour average dosage was 2 to 3 ppm benzene; the average concentration determined by 15-minute sampling was 25 ppm, and peak concentrations were 50 ppm (as a consequence of certain specific operations such as sampling and repairs). Exposures could reach 100 ppm if the employee failed to stand upwind while collecting samples. This is a carefully conducted investigation, and the results appear to be significant.

Picciano,[48] who was involved in the Dow study, using the same data, records a dose-response curve at benzene levels of less than 1 ppm. His report, as well as that presented by Dow,[32] indicated a 10-fold increase (compared with controls) in the percentage of benzene workers exhibiting chromosome breaks and "marker" chromosomes (dicentric chromosomes, translocations, and exchange figures).

Most of these industrial studies were systematic to some degree, including controls and statistical evaluations of results. These studies of workers from several European countries all present similar results; that is, statistically significant increases in both numerical and structural chromosome alterations in populations exposed to benzene. PHA-stimulated lymphocytes showed both stable and unstable chromosome changes in the absence of detectable alterations of the bone marrow, and aneuploidy or polyploidy was reported frequently. In studies where little or no clinical symptomatology resulted from exposure, there was considerable variation among individuals. For example, in Girard's[19] and Forni's[17] studies a few individuals within each benzene-exposed group were responsible for the significantly higher chromosome breakage rates in the exposed populations. Moreover, it is clear that these changes persisted for many years after exposure, particularly in persons who showed clear evidence of clinical illness from benzene. The persistence of damage has been likened to that occurring after exposure to ionizing radiation. The few reported instances of abnormal clone formation[18,51] are important in terms of possible leukemogenesis. There is no correlation, however, between the degree or length of exposure to benzene, the clinical symptoms, and the persistence or extent of chromosomal aberrations.

Embryonic and Teratogenic Effects

The literature contains few reports concerning the effects of benzene on embryogenesis. In one study,[20] groups of female rats were exposed continuously for 10 to 15 days prior to mpregnation to concentrations of benzene vapor that ranged from 0.3 to 209.7 ppm. In the animals exposed to 209.7 ppm no pregnancy occurred. In the animals exposed to 19.8 ppm, resorption of embryos was observed in one of ten. The number of offspring per female exhibited an inverse relationship to benzene exposure levels. The weights of the newborn rats did not vary from those of the controls, but the ratio of organ weight to body weight in the 19.8-ppm exposure group was significantly higher than that in the controls (liver, lung, spleen, and kidney). A study conducted by Litton Bionetics, Inc.,[3] indicated a small increase in the frequency of fetal resorptions in rats exposed to 10 to 40 ppm benzene during days 6 to 15 of gestation. The incidence of resorptions was too low to allow firm conclusions. Another study with concentrations of benzene at 2200, 300, and 100 ppm showed no evidence of increased fetal resorption.[22] In sum, the reports of effects of benzene on embryos are conflicting and inconclusive and hence are not useful in evaluating the possibility that low, ambient concentrations of benzene might have an effect on human embryogenesis.

In another study,[68] groups of pregnant mice were given a single very high subcutaneous injection of benzene (3 ml/kilogram body weight) on days 11 to 15 of gestation and the fetuses were delivered by caesarian section on day 19. Malformations occurred more frequently in fetuses of mice injected on day 13 than on other days, and the anomalies included cleft palate, agnathia, and micrognathia.[68] Greene et al[22] reported that at dosages of 2200 and 300 ppm benzene, skeletal deformities (missing sternebra) were observed. Since reports of effects of benzene on teratogenesis are few and the concentrations of benzene used are very high, a role for benzene in teratogenesis cannot be predicated with confidence at this time.

Summary

The available documentation strongly suggests that chromosome breakage and rearrangement can result from exposure to benzene and that damage may persist in hematopoietic and lymphoid cells. The aberrations in human cells appear nonspecific; that is, they are random within the genome and unrelated to the aberrations associated with various forms of leukemia. A dose-dependent relationship between exposure to benzene and amount of chromosome damage has not been demonstrated. Evidence that benzene causes disturbance in DNA synthesis suggests that its mutagenic action could involve interference with mitosis. Cytogenetic analysis of anaphase and postmitotic damage has not been evaluated adequately.

Theoretical considerations and some clinical observations suggest a relationship between chronic benzene exposure, chromosome damage, and leukemia. Chromosomally aberrant clones are typical of some but not all human leukemias, and aberrant cells and clones have been observed in individuals exposed to benzene who have later developed leukemia. Many authors have suggested that the lack of an observed dose-response relation in benzene-induced chromosome damage is due to variation in individual susceptibility. Some studies have recorded biological effects at (chronic) exposure levels below 25 ppm. The report of a recent international workshop on the toxicology of benzene has commented on this literature: "No dose-effect relationship has so far been demonstrated for benzene-induced chromosome aberrations. In workers chronically exposed to levels in the range of 5 to 25 ppm of benzene, both positive and negative reports involve small numbers of workers and confirmation of negative data is required on larger groups." Increased susceptibility to chemical clastogens has been found in human cancer syndromes that are genetically determined.[6] The variable response to benzene may be attributed also to such possibilities as activation of virus, suppression of immune surveillance, or cocarcinogenic activity of other chemicals.

More detailed evaluation of the cytogenetic effects of benzene will require definitive data on dose/response relationships, relating the frequency and severity of chromosome damage to the amount and duration of benzene exposure. Both clastogenic and antimitotic measures of chromosomal mutagenicity should be evaluated. Benzene dosage should be correlated with clinical effects as well as with the various measures of chromosome damage. When an appropriate animal model becomes available, the evolution and sequence of chromosome changes with initiation and progression of leukemia may become clear.

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SECTION 3

CHRONIC BENZENE TOXICITY IN ANIMALS

The earliest report of experimental work on chronic benzene toxicity was published by Santesson[32] in 1897. He had been asked to determine the toxic agent in an industrial solvent that had caused purpura hemorrhagica in a group of female employees. He succeeded in producing similar effects in rabbits after treating them with benzene by poultice and by subcutaneous injection.

Selling,[35] who had studied several cases of benzene-induced purpura hemorrhagica, leukopenia, and anemia in young girls exposed to benzene in a canning factory, extended his investigation of the disease to rabbits. He administered benzene daily by subcutaneous injection and demonstrated a dramatic decrease in leukocytes, a smaller decrease in red cells, and degenerative changes in bone marrow. This study was a landmark because it was the first in which the investigators measured the depleting effect of benzene on numbers of circulating blood cells and related the decreases to bone marrow damage.

In subsequent studies of benzene toxicity, the solvent either has been administered as a vapor, which the animals inhale in an exposure chamber, or has been injected as the pure solvent or as a mixture with a carrier such as oil. The injections are often subcutaneous. The following discussion focuses first on experiments with inhalation, which is the most common route of industrial exposure, then compares results of exposures by inhalation with those resulting from injection of benzene.

EXPOSURES BY INHALATION

Santesson[32] and Selling[35] were not successful in attempts to produce benzene toxicity by the inhalation route. Weiskotten,[45] who exposed rabbits to benzene at a concentration calculated to be approximately 240 ppm,[37] produced leukopenia, slight anemia, and hemorrhages. Differential blood cell counts suggested that small mononuclear white cells were depressed more than "polynuclear amphophiles." The effects were observed within 2 weeks of exposures for 10 hours per day.

Svirbely et al[40] exposed rats, dogs, and mice to 1000 ppm of benzene 7 hours per day, 5 days per week for 28 weeks. Although measurements indicated intermittent leukopenia and lymphocytopenia in the rats, the values returned to control levels by the time the experiment ended. The dogs demonstrated lymphocytopenia throughout the study. Hough et al[13] exposed nine dogs to a mean concentration of 800 ppm benzene for 4782 hours over a 123-week period and observed a decrease in leukocyte counts from 15,917 ± 2863 (M±SD)* to 6272 ± 3481, i.e., a reduction to 39 percent of control values. Differential cell counts were not performed, and no anemia was observed.

Li et al[24] investigated the effects of varying the protein and fat contents of the diet of dogs on their susceptibility to the depressant action of benzene on bone marrow. The dogs were fed equicaloric diets described as 1) low fat, high protein; 2) high fat, high protein; 3) low fat, low protein; and 4) high fat, low protein. Controls included a group fed high fat, no protein and a group that was not exposed to benzene and was fed high fat, low protein. The exposed animals inhaled benzene at 600 ppm for 42 hours per week over varying periods of time depending on their responses to benzene. Those least affected were continually exposed for periods longer than 1 year, whereas others were sacrificed when they became moribund after periods as short as 5 to 6 weeks. Monitoring of leukocyte and thrombocyte levels indicated that benzene exposure reduced the numbers of both types of cells but that protein deficiency produced greater reductions, which were in turn exacerbated by high-fat diets.

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* Mean value plus or minus the standard deviation.

In a complex series of studies, Wolf et al[46] used 7-hour exposure periods. They exposed rats (9400 ppm, 1 to 10 times over 1 to 19 days; 6600 ppm, 70 times over 93 days; 4400 ppm, 28 times over 38 days; 2200 ppm, 133 times over 212 days; 88 ppm, 136 times over 204 days), guinea pigs (two studies: 88 ppm, 193 times over 269 days; 99 ppm, 23 times over 32 days) and rabbits (80 ppm, 175 times over 243 days). Although details of cell counts were not reported, the authors claim to have observed leukopenia in animals exposed to levels as low as 80 ppm and also to have observed histopathological changes in bone marrow.

Deichmann et al[5] exposed rats to benzene at various doses, usually for 5 hours per day, 4 days per week. A decrease in white cells was observed at benzene concentrations of 831, 65, 61, 47, and 44 ppm over periods ranging from 2 to 8 weeks. No leukopenia was observed at concentrations of 31, 29, or 15 ppm. Females were more sensitive than males, but differential counts were not reported. Splenic hemosiderosis was a prominent, but not dose-related, observation.

Nau et al[27] exposed rats to benzene at three dose levels (1000, 200, and 50 ppm) for various periods of time. At 1000 ppm, rats exposed for 23.5 hours per day seriously deteriorated after 183 hours, showing distended stomach, empty gastrointestinal tract, and engorgement of lungs, liver, kidneys, intestines, and omental tissues. In addition, the leukocyte count dropped markedly. Lymphocyte levels appeared to decrease, while polymorphonuclear leukocytes increased. Levels of DNA in the bone marrow were depressed, and the proportion of red cell precursors was increased. When exposure was reduced to 19 hours per day for up to 1782 hours, similar effects were observed. When the rats were removed from benzene exposure, the blood analyses showed a return to normal, except that levels of DNA in the bone marrow remained depressed. In exposures at 200 ppm for 8 hours per day, 5 days per week, leukopenia occurred after 750 hours, with equal reduction in lymphocytes and polymorphs. Myelocytic activity of the bone marrow was reduced, and erythroid activity was increased. Similar effects were observed after exposures at 50 ppm.

Jenkins et al[17] detected no significant changes in leukocyte, hemoglobin, or hematocrit values in rats, guinea pigs, or dogs exposed to benzene concentrations of 255, 30, or 17.5 ppm during repeated exposures for 8 hours per day for 30 days or during continuous exposures for 90 or 127 days.

Ikeda[15] exposed rats to benzene concentrations of 1000 ppm for 60 days, 7 hours per day, 5 days per week. These exposures indicate that age and sex may affect susceptibility to benzene toxicity in rats, since the leukocyte levels decreased in the following order: adult males, young males, adult females, young females. Both Ikeda and Ohtsuji[16] and Drew et al[8] reported that pretreatment of rats with phenobarbital protected against depression of leukocyte levels during exposure to atmospheric benzene at levels of 1000 ppm and 1650 ppm, respectively. Boje et al[2] exposed rats to 400 ppm of benzene 7 hours per day for 13 weeks and observed marked leukopenia. Radioautography of samples of bone marrow from benzene-exposed rats given tritiated thymidine showed that benzene-treated animals displayed less incorporation of radioactivity than did control animals. These authors hesitated to suggest a direct inhibition of DNA synthesis by benzene because they recognized that cellular damage occurring at any point in the cell cycle might be manifested as a reduction in thymidine uptake.

Uyeki et al[41] studied two parameters of benzene toxicity not previously reported. The colony forming cell (CFC) assay is a measure of granulocyte precursor activity of the bone marrow. In these studies, the assay involved culturing marrow cells from benzene-treated mice in an appropriate medium. The authors used the number of colonies of granulocytic cells formed as a measure of the number of granulocyte precursors in the marrow. The colony forming unit (CFU) assay, which measures stem cells, also was used in these studies. The assay involved injecting marrow cells from benzene-treated animals into X-irradiated mice and evaluating the subsequent formation of colonies on the spleen of the receptor mouse. One day after the mice were exposed to 4680 ppm of benzene for 8 hours, both CFC and CFU activity were reduced to 40 to 45 percent of that measured in controls. Repeated exposure by inhalation further reduced CFC activity.

The most recent studies on inhalation of benzene in rats and mice, performed over a 2-year period by Laskin et al,[10] are as yet unpublished. Mice and rats were exposed to benzene at 100 and 300 ppm and were examined periodically for signs of change in blood cell levels and for indication of aplastic anemia and/or leukemia. Although evaluation of these data is not yet complete, some observations may be made. Although depressions of white cell levels were observed and animals that died during the study often appeared to undergo depletion of bone marrow, other animals that died displayed no signs of bone marrow toxicity. Of special interest is the observation that decreased levels of white cells generally were reflective of lymphocytopenia but not of granulocytopenia. Further evaluation of the data is ongoing, and additional useful information is expected. Preliminary assessment indicates that in C-57 Black mice and Sprague-Dawley rats that were exposed to 300 ppm of benzene for approximately 1 year, lymphocyte counts were reduced to 25 and 60 percent of those in controls, respectively, during the first 5 to 10 weeks of exposure. The counts remained close to those levels for the remainder of the year. The mice, but not the rats, also displayed a reduction in erythrocyte counts, which became apparent at the same time as the lymphocytopenia and remained fairly constant at about 68 percent of control levels throughout the remainder of the period.

EXPOSURE BY OTHER ROUTES

Both Santesson[32] and Selling,[35] administering benzene subcutaneously as described above, produced benzene toxicity. Selling administered the benzene dissolved in olive oil to rabbits at a dose of 1 ml/kg per day. He monitored leukocytes until they were reduced to a level of 200 to 800 cells per mm^3. Because of differential sensitivity among the animals, periods of 4 to 9 days were required to reach these low levels. When additional injections were given, most of the animals died. Since these experiments were of short duration, red cell levels did not vary significantly. In a similar series of studies Weiskotten et al[43] treated the animals until the white cell count dropped to 1000 per mm^3. Studies of recovery after cessation of treatment by Selling,[35] Weiskotten et al,[44] Brandino,[3] and Secchi[34] showed that the white cell counts initially rise, then go through a secondary depression, which Weiskotten described as the deuterophase, and then return gradually to control values. It is emphasized that Selling[35] produced complete aplasia of the bone marrow when giving benzene parenterally, an effect that corresponds well with the observation of aplastic anemia in humans exposed via inhalation to benzene in industrial environments.

Gerarde[9] performed a similar set of experiments in which rats were treated daily for 2 weeks with 1 ml/kg of benzene as a 50 percent solution in olive oil. He continued to observe the animals for an additional 3-week period, during which treatments were discontinued. Leukopenia was observed during the initial 2-week treatment period and was accompanied by decreases in the levels of nucleic acid and of nucleated cells in the marrow of the femur. When administration of benzene was discontinued, each of these three parameters returned to normal. Latta and Davies[22] treated rats with a solution of 50 percent benzene in olive oil in doses of 2, 3, and 4 ml/kg daily for up to 60 days. Following a temporary stimulation of white cell production, the circulating leukocyte levels dropped and the data suggested impairment of leukocyte maturation. Lymphocyte production appeared to be more dramatically impaired than was granulocyte production. At the higher doses no myelocytes were observed after 24 days, and the marrow was completely aplastic after 60 days.

Steinberg[39] observed degeneration of bone marrow in benzene-treated rabbits. This was followed by regeneration after the benzene treatments were stopped. Although extrapolation of doses from his data is difficult because the weights of the animals are not given, it is calculated that the dose was approximately 2 ml of benzene per day per rabbit. The animals were dosed subcutaneously for 6 to 70 days. Under these conditions leukocyte counts were reduced but not in a dose-related fashion. Throughout the treatment period white cell counts in the dosed animals averaged 22.6 ± 7.3 percent of control counts, the calculation being based on all values from day 6 through day 70. As part of these studies, Steinberg determined the ability of the marrow to regenerate by comparing regeneration in rabbits from which marrow had been surgically extracted with regeneration in rabbits that had been intoxicated with benzene. Unlike control animals, benzene-treated animals displayed regeneration only to the extent of formation of primitive reticular cells.

Nomiyama,[28,29] who performed daily subcutaneous injections of rats with benzene (1 gm/kg), reported reduction of leukocyte counts. In similar studies with several strains of mouse, he found a strain-dependent variation in sensitivity to benzene.

Speck et al[19,20,26] administered benzene to rabbits at doses of 0.2 and 0.3 ml/kg per day and produced severe leukopenia within 1 to 9 weeks. Although reductions in hemoglobin and red cells were more pronounced, reductions in reticulocytes and thrombocytes also were observed. Pancytopenia was a common finding. Among 19 marrow smears, 21 percent were very hypoplastic, 32 percent were hypoplastic, 26 percent appeared normal, and 21 percent were hypercellular. No correlation between cellularity and duration of exposure to benzene was apparent. In general, myeloid precursors were diminished more than erythroid cells. After treatment of the animals with tritiated thymidine, radioautography of the marrow indicated that incorporation of radioactivity into DNA was reduced. The authors interpreted these findings to indicate that benzene toxicity leads to a decrease in DNA synthesis. These authors also demonstrated chromosomal aberrations in rabbits similarly treated with benzene. Finally, they reported a decrease in incorporation of tritiated cytidine into RNA, which they interpreted as an inhibition of RNA synthesis.

Lee et al[23] demonstrated that benzene depressed the incorporation of a radioactive iron isotope (^{59}Fe) into circulating erythrocytes. Radioactivity from ^{59}Fe disappears from the blood soon after parenteral administration and reappears when incorporated into hemoglobin. The rate of reappearance reflects the maturation and proliferation of the components of bone marrow cells. Single doses of benzene in corn oil at 400 mg/kg and 2200 mg/kg were given to mice. In measurements of 24-hour uptake, the reappearance of radioactivity reflects only reticulocyte activity, whereas measurements of 72-hour uptake reflect the entire cycle from stem cell to mature red cells. Thus, the 24-hour uptakes that were measured 24 or 48 hours after giving benzene were lower than those of controls, whereas they were not lower when iron was given 1, 12, or 72 hours after administration of benzene. Based on current knowledge of red cell maturation in the mouse, these data suggest that the cells most sensitive to benzene are early precursors called pronormoblasts and normoblasts. In similar experiments in which 72-hour uptakes were measured, the reductions in iron utilization were less dramatic, perhaps because the longer uptake period allowed sufficient time for compensatory mechanisms to come into play.

In more recent studies[38] not yet published, Lee et al administered benzene to mice in multiple doses over several days. They found that when mice were given a single subcutaneous dose (440 mg/kg) of benzene per day, incorporation of ^{59}Fe was reduced 50 to 60 percent in 20 days. When mice were given 880 mg/kg in single daily doses, iron utilization decreased gradually to 80 percent of controls in 10 days. When doses of 440 mg/kg were given twice per day (i.e., a total daily dose of 880 mg/kg given as two doses) the reduction in iron uptake occurred more rapidly, and by the tenth day almost no iron was taken up. Finally, when two doses of benzene, at 880 mg/kg per dose, were given each day, incorporation of iron appeared to terminate by the sixth day. These data suggest that a dose-times-time relationship governs the cumulative effects of benzene. Giving the dose twice per day (440 mg/kg each) exacerbated the effect over that observed when the same amount was given as a single dose, perhaps because the single dose schedule allows for essentially complete excretion of the benzene either as free benzene in exhaled air or as water-soluble metabolites in the urine. When the dose is divided, the animals apparently retain some benzene the morning after, to which the next dose adds. Therefore, the animals accumulate more and more benzene as the experiment progresses.

The oral route is the least investigated in studies of benzene toxicity. Wolf et al[46] administered 132 feedings of benzene to rats at doses of 1, 10, 50, and 100 mg/kg over a 187-day period. No effect was observed after the 1 mg/kg dose, slight leukopenia was observed after the 10 mg/kg dose, and both leukopenia and anemia were observed after the higher doses.

EVALUATION AND COMMENTS

The major problem in extrapolation of data obtained in animal studies to benzene toxicity in humans relates to deficiencies in use of controls and in blood counting techniques in the animal studies. Normal values of total leukocytes in common laboratory animals such as the mouse, rat, and rabbit range widely from approximately 4000 to 5000 cells per mm^3 to over 20,000, with occasional values in the rabbit as low as 3200.[33] Thus, it may be argued that reductions in white cell count within that range may not reflect true toxicity but may reflect successful protective responses by the organism. In contrast, decreases in leukocyte levels to below 2000 cells per mm^3 may reflect insults severe enough to impair the health of the animal. In many reports that claim the production of leukopenia, however, it is implied that leukopenia is defined as a reduction of white cell levels below some control value. When the control values are set by measurements of the test animals prior to exposure, an error may be introduced because of natural variations in white cell counts. For example, Cheng[4] reported that leukocyte counts in rabbits increase gradually from 2000 in the first week of life to between 4500 and 6000 cells at 100 to 200 days of life. Considerable variability was observed among 240 adult rabbits in which white blood cell (WBC) counts averaged 7000 ± 5000 cells. Although age may not be a factor in studies with mice, the time of day of sampling, the site from which the sample is taken, and the strain of mouse all affect the cell count.[33] The effect of age on the leukocyte count in rats remains an open question. Reich and Dunning[31] studied eight different strains of rat, and Harris and Burke[11] studied the Wistar rat, a strain not examined by Reich and Dunning. Both groups reported that age did not affect leukocyte counts. It was suggested that the neutrophil-lymphocyte ratio increases with age in rats. Recently, Laskin et al[10] found that a relative decrease in leukocytes occurs within the first 3 months of life of Sprague-Dawley rats. These observations indicate that claims for benzene-induced leukopenia should be supported by proper controls for age, as well as for exposure.

Although the extreme decreases in leukocytes observed by Selling,[35] Weiskotten and co-workers,[44] and Kissling and Speck[20] clearly demonstrated leukopenia, the studies of Hough et al,[13] Wolf et al,[46] Deichmann et al,[5] Nau et al,[27] Ikeda et al,[15,16] Drew et al,[7] and others must be evaluated with respect to the age effect and other factors.

A second question regarding changes in white cell counts concerns the interpretation that reductions in numbers of white cell must, of necessity, reflect bone marrow damage. Relatively few investigators have performed differential counts of the white blood cells. Laskin et al[10] have shown that during extended exposure to benzene, the total WBC counts in rats and mice decreased but the numbers of granulocytes apparently did not. Since granulocytopenia reflects bone marrow damage, these animal models may not reflect the human disease in which granulocytopenia is a prominent feature.

Even with proper controls and differential cell counts, however, proper interpretation of the effects of benzene in animal experiments will be difficult if evaluation of peripheral blood factors remains the sole focus of attention. The problem can be exemplified by comparing the results of Speck and associates[19,20] with those of Boje et al.[2] The Speck group treated rabbits with benzene subcutaneously until white cell levels decreased below 1000 per mm^3. Under these conditions mitosis in bone marrow was impaired and uptake of tritiated thymidine into DNA was reduced significantly. When Boje et al[2] exposed rats to benzene by inhalation at 400 ppm for up to 13 weeks, they demonstrated a similar decrease in DNA synthesis in the bone marrow, but in this case the white cell levels were decreased to only 46 percent of controls, which represents a considerably higher count than Speck's 1000 per mm^3. Thus, significant damage to marrow may occur when leukocyte levels remain relatively high. It must be argued then that reductions in white cell levels, even when the counts remain in the normal range, may be indicative of marrow damage and it is not necessary to demonstrate leukocyte levels below 2000 cells to suspect benzene-induced marrow damage. Careful studies of the effects of benzene on circulating blood cell elements should be supported with information concerning the effects of benzene on bone marrow.

Benzene and Leukemia in Animals

Leukemia is a general term for a group of diseases usually characterized by large increases in numbers of white blood cells in blood and/or bone marrow or the appearance of unusual leukocyte precursors in the blood. Leukemia is associated with neoplasms, i.e., new growths of tissue serving no physiologic function. It is usually accompanied by a variety of defects in the hematopoietic, or blood-cell forming, system. Leukemias are known to occur spontaneously in some strains of mice, and there is abundant evidence that chemicals can induce leukemia in both mice and rats.[6,12,14,25,30,36] Lignac[25] reportedly produced leukemia in mice by treating them with benzene subcutaneously for 17 to 21 weeks. Of the 44 mice that survived treatment, 8 were described as having developed leukemia or lymphosarcoma. Failure to include control mice and to provide details concerning the strain of mouse and the diagnostic criteria leaves this report open to question.

In a specific attempt to duplicate the results of Lignac, Amiel[1] treated mice of the AKR, DBA_2, C_3H, and C57Bl strains with weekly injections of benzene (30 mg/kg) throughout their lifetimes but observed neither aplastic anemia nor leukemia. Ward et al[42] studied a group of C57Bl mice for a total of 104 weeks, during which time the dosage schedule was varied but in general was increased from 450 mg/kg to 1.8 gm/kg. Although a number of mice died of the toxic effects of benzene, there was not a statistically significant increase in incidence of neoplastic disease in benzene-treated mice over that observed in controls.

The evidence for production of leukemia in animals by injection with benzene must be considered nonconclusive. Moreover, neither oral dosing,[46] skin painting with benzene,[18,21] nor inhalation[17,46] had been demonstrated to produce leukemia or any other type of neoplastic disease in rats, mice, guinea pigs, or rabbits. Suggestive results, however, have been reported very recently by Nelson*. Of 40 mice (strain CD-1) exposed over their lifetimes to 300 ppm benzene, 2 died of leukemia (1 with chronic myelogenous leukemia, 1 with acute stem cell leukemia of possible myeloblastic type). Of 45 Sprague Dawley rats exposed similarly to 100 ppm benzene, 1 died of chronic granulocytic leukemia. There have been no published reports of spontaneous cases of myelogenous leukemia, either acute or chronic, in CD-1 mice or Sprague-Dawley rats.

These findings, although the experiments were performed with relatively small numbers of animals and at moderately high doses of benzene, are consistent with epidemiological data in humans indicating a relationship between benzene inhalation and leukemogenesis. The results, if confirmed, may indicate the possibility of the much-desired animal model for leukemogenesis.

In contrast, with respect to possible benzene-associated leukemia in humans, evidence from industries that have used benzene heavily implies a direct relationship between benzene exposure and development of leukemias. Furthermore, the leukemias have been observed mainly in cohorts of workers among whom many showed signs of benzene-induced bone marrow damage of variable severity. Despite the inability of investigators to demonstrate leukemia in groups of animals exhibiting bone marrow damage, the studies of humans provide compelling evidence that benzene is involved in leukemogenesis.

Several theories might be offered to explain the inability of researchers to induce leukemia in animals by treatment with benzene. Man may be the only species yet observed that is susceptible to benzene-induced leukemia for a variety of reasons such as 1) a novel metabolic pathway that produces a unique reactive metabolite not formed in other animals, 2) relative inefficiency in repair of DNA of benzene-induced damage, or 3) relative ineffectiveness of immune surveillance following benzene-induced insult. Also, the phenomenon may involve other mechanisms of which we are not aware. It is possible that the latency period in animals is long enough to preclude the appearance of leukemia during the lifetime of the animal, or an as-yet-unknown cocarcinogen may be required to evoke the leukemogenic response initiated by benzene. If bone marrow damage is a prerequisite for benzene-induced leukemia, the appropriate animal experiments may not have been done. For example, it may be necessary to induce bone marrow damage and then to allow for recovery during the remainder of the life of the animal. Regular monitoring might then show some animals with leukemia. The researcher then should demonstrate that a similar control population displays significantly fewer cases of leukemia. In any attempts to disclose a cause and effect relationship between benzene exposure and leukemia, it is essential that the studies include sufficient numbers of control and treated animals. The need for large numbers of animal subjects is underscored by the exceedingly low incidence of benzene-induced leukemia in human workers. Meaningful statistics on these cases have been collected only when large groups of workers were observed.

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*Nelson, N. Letter to R. Cortesi, EPA, March 13, 1978.

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SECTION 4
BENZENE TOXICITY IN MAN

INTRODUCTION

The world medical literature contains hundreds of references describing thousands of cases of human hematological toxicity associated with benzene exposure. This literature has been reviewed relatively recently by a number of authors.[24,39,78,88,145,148,172] Although numerous hematological disorders have been reported in association with benzene exposure, only two entities are clearly related to benzene. These are 1) pancytopenia and its variants, including anemia, leukopenia, thrombocytopenia and aplastic anemia; and 2) acute myelogenous leukemia and its variants, such as acute myelomonocytic leukemia and erythroleukemia. Unfortunately, there is a relative paucity of information concerning the doses of benzene to which affected individuals were exposed.

This section deals with the evidence presented in the literature concerning the human hematological toxicity of benzene. The emphasis is primarily on studies that have evaluated relatively large numbers of occupationally exposed individuals, particularly where measurement of dose has been attempted. Most of the rest of the reports describe one or a few cases of hematotoxicity associated with benzene exposure. Such reports do have cumulative weight, particularly in relation to the causative role of benzene in acute myelogenous leukemia. Individually, however, they provide little information not given elsewhere and therefore they are not discussed here.

In the reported cases of benzene hematotoxicity, exposure has usually occurred in a workplace where benzene was used as a solvent or manufactured as a product. In some instances, benzene was used because it is inexpensive and has excellent solvent properties. In other instances, benzene was an inadvertent contaminant of aromatic hydrocarbon solvents. Whatever the reason for its use, in almost all cases in which benzene hematotoxicity has been reported, the patient has been exposed also to some other solvent or chemical product. That it is benzene, rather than some other commonly associated agent such as xylene or toluene, that is primarily responsible for hematotoxicity appears well established. The major evidence supporting this assertion includes the observation that benzene has been the common denominator in many different occupational settings in various parts of the world in which exposure to other chemicals has varied widely. In addition, the pancytopenic effect of benzene in man can be readily duplicated in experiments with a variety of animal species. This is not true of the other commonly associated contaminants.

It is possible, however, that compounds inhaled along with benzene alter the expression of benzene hematotoxicity. In particular, it is conceivable that other aromatic hydrocarbons may modify benzene metabolism in humans. This is an area in which additional information on the effects of benzene in man would be of great value.

PANCYTOPENIA

The term pancytopenia refers to a diminution of all formed elements in the blood. In benzene exposure this diminution is due primarily to an interference in the production of red cells, white cells, and platelets in the bone marrow, although some evidence suggests that survival of blood cells within the circulation may be shortened also. Many of the cases associated with benzene exposure are described as "aplastic anemia." This term classically denotes a condition in which the number of hematopoietic precursor cells within the bone marrow diminishes markedly. Aplastic anemia is usually associated with a high degree of pancytopenia and is observed particularly in severe or fatal cases. However, a decrease in bone marrow cellularity is not always noted in individuals with hematotoxicity associated with benzene exposure nor in all animals treated with benzene. This may be attributable to individual variation, to ineffective response of poietic bone marrow to benzene, or to the sampling error inherent in the presumption that a local aspirate of bone marrow represents all hematopoietic tissue. For purposes of discussion, cases of clear bone marrow aplasia are considered under the heading of pancytopenia.

Numerous cases of individual cytopenias, e.g., anemia without leukopenia or thrombocytopenia, also are reported in benzene-exposed individuals. These have been observed mostly in industries where severe benzene hematotoxicity in some workers has led to evaluation of the entire work force. It should be noted that there is wide variation in the normal red cell, white cell, and platelet counts as well as appreciable reserve production capability. A person in whom one of the formed elements decreases to a level below the accepted normal value may also experience an effect on the production of other cell types that is not clinically apparent. Accordingly, the individual cytopenias are also discussed under the heading of pancytopenia.

Exposure to benzene clearly produces hematological toxicity in animals and man. Evidence of a pancytopenic effect of benzene was first noted in 1897 by Santesson, who reported four cases of fatal aplastic anemia occurring in workers fabricating bicycle tires. Since that time numerous case reports and surveys of occupationally exposed groups of workers have documented this association, and many reviews of these cases have appeared.[24,39,78,85,88,92,145,148,165,172,173] The causal relationship of benzene to pancytopenia in man is most clearly supported by studies, described below, of groups of workers in whom the appearance of pancytopenia was temporally related to the inception of benzene use and in whom the outbreak of hematological effects was ended by replacement of benzene with some other solvents.

Current concepts of the hematopoietic system stress the role of a pluripotential myeloproliferative stem cell in production of erythrocytes, granulocytes, platelets, and perhaps fibroblasts and other monocytic cells. This stem cell is believed to be able to differentiate into the precursors of the various formed elements in response to microenvironmental conditions. The production of pancytopenia suggests that benzene is toxic to this stem cell or to early hematopoietic precursors of red cells, granulocytes, and platelets. Some incomplete evidence also points to an even earlier pluripotential cell with capabilities of differentiating into lymphocytic as well as myelocytic precursors. This may be pertinent to benzene toxicity in that lymphocytopenia is frequently observed in pancytopenic individuals with a history of benzene exposure. In addition, chromosomal abnormalities in circulating lymphocytes have been reported in association with benzene exposure (see Section 2), and evidence suggests that such exposure increases the risk of lymphocytic neoplasms.

The symptoms of pancytopenia in individuals exposed to benzene are described by a number of authors who have investigated occupationally exposed groups. In milder cases, these tend to be such nonspecific complaints as lassitude, tiredness, easy fatigability, malaise, dizziness, headaches, palpitation, and shortness of breath. Such symptoms tend to appear gradually, and presumably reflect anemia. Occasionally, in more severe cases, hemorrhagic manifestations due to thrombocythemia or decreased platelet function are also observed. In severe cases of pancytopenia, death is often due to hemorrhage or to overwhelming infection, the latter reflecting the decrease in granulocytes. In addition, patients with pancytopenia may subsequently develop fatal acute leukemia, sometimes with an intervening period of apparent recovery.

Among the earliest systematic evaluations of the pancytopenic effects of benzene are those of Greenburg et al,[82] Goldwater,[79] and Goldwater and Tewksbury.[80] These investigators studied workers in rotogravure printing plants where benzene had been in use for a period of 3 to 5 years. The benzene contents of the ink solvents and thinners ranged from 10 to 80 percent. Forty-eight analyses of benzene concentrations in the air of pressrooms of three plants revealed levels ranging from 11 to 1060 ppm, with a median concentration of 132 ppm. The most frequent clinical complaints were fatigue, dryness of the mucous membranes, lethargy, dizziness, headache, and shortness of breath. Hematological studies were performed in 332 exposed male workers and 81 controls. Various degress of hematological toxicity were observed in 65 of these workers, 23 of whom were considered to be severely affected. As a result of these studies, six individuals were referred for hospitalization. The remaining workers continued on the job and, following replacement of benzene with other solvents, hematological recovery was demonstrated.[80] In comparison with the control group, the most frequently observed findings were anemia, macrocytosis, and thrombocytopenia. An absolute lymphocytopenia was more common than was neutropenia, which was rarely observed. Other relatively infrequent findings were prolongation of the bleeding or coagulation times, an increase in capillary fragility, and an increase in serum bilirubin and reticulocytes. Osmotic fragility was normal, as was the erythrocyte sedimentation rate. No monocytosis, eosinophilia, or basophilia was observed.

Several occupationally exposed groups were studied during World War II. Wilson[195] evaluated workers in an American rubber factory in which peak benzene levels were said to be 500 ppm, with an average of about 100 ppm. Of 1104 workers studied, 83 were observed to have mild hematological effects and 25 had more severe pancytopenia. Nine of the latter group were hospitalized, and three died. Hamilton-Patterson and Browning[86] reported observations of 200 women in 13 aircraft factories in England as compared to 200 controls. These workers had been involved in rubber manufacture and had been exposed to solvents and adhesives containing 5 to 20 percent aromatic hydrocarbons. In contrast to the findings of Greenberg et al[82] and Goldwater[79] these authors suggest that neutropenia is the earliest and most consistent indicator of benzene toxicity. No significant difference in red cell or lymphocyte counts was observed. Helmer[89] in Sweden evaluated 184 workers (169 women, 15 men) in a rubber raincoat factory where benzene levels of 137 to 218 ppm were measured and it was believed that levels had been higher in the past. Evidence of hematological toxicity was observed in 60 individuals (58 women, 2 men). Reevaluation 16 months after cessation of benzene use revealed that 46 recovered, 12 still had significant effects, and 2 had died. The most frequent symptoms were gradual development of headache and tiredness. The author also notes the frequency of cutaneous hemorrhages and stresses the finding of thrombocytopenia. Hutchings et al[93] studied 87 benzene-exposed individuals in Australian air force workshops after the discovery of a fatal case of aplastic anemia. The measured peak benzene concentrations ranged from 10 to 1400 ppm, and were well above 100 ppm in most areas. For most of the time, however, the concentrations ranged from 10 to 35 ppm. Solvents contained up to 53 percent benzene. In addition, the worker exposure time was studied in relation to atmospheric benzene concentrations.

Little difference was observed in the hematological measurements of 87 benzene-exposed workers as compared to those of 500 workers exposed to other hydrocarbons and 300 unexposed controls, although there was a tendency toward a lower hemoglobin and platelet counts. The duration of benzene exposure in these workers is unclear. Hutchings et al conclude that the worker who died of aplastic anemia may have been unusually susceptible to the effects of benzene.

Pagnotto et al[140] report a study of benzene exposure in a rubber coating plant where petroleum naphtha containing 1.5 to 9.3 percent benzene was in use. Atmospheric benzene levels were generally less than 25 ppm but ranged up to 125 ppm. Correlation between urinary phenol levels and measured concentrations of benzene in air was excellent. In one plant, the hemoglobin levels of 5 of 32 men studied were reduced. One of these men had the second highest urinary phenol level measured in the plant (480 mg per liter, equivalent to 58 ppm benzene exposure). In a second plant, only 1 of 9 individuals studied was anemic, but this worker was severely affected requiring hospitalization. In a third plant, none of six workers studied was anemic, nor was leukopenia observed. Some follow-up data suggesting mild persistent anemia are presented by the National Institutes of Occupational Safety and Health (NIOSH).[145]

Several studies of groups occupationally exposed to benzene have been reported in the past decade. Stewart et al[174] (published in abstract only) reported on ten persons with mild anemia and macrocytosis who had recovered within 8 months following apparent cessation of benzene exposure. Kliche et al[104] reported studies of 18 roof tilers exposed for an average of 17 years to what is stated to be 15 ppm benzene. Six of these persons are described as having mild early disease and seven as having chronic benzene hematotoxicity. The author also presents indirect evidence of qualitative abnormalities of platelets despite normal platelet counts in these subjects.

A number of Eastern European studies of benzene-exposed workers have been reported in recent years. Doskin[53] evaluated 365 workers employed for 3 years in an apparently new chemical factory. Although detailed monitoring apparently was performed, the measured concentrations are not given. The author states that benzene levels exceeded the maximum permissible concentration 2 to 8 fold in 64 percent of the measurements in the first year, 37 percent in the second year, and 3 percent in the third year. The analytical method and total number of measurements are not stated. We believe, although we have not been able to confirm, that the maximum permissible concentration of benzene in the Soviet Union at that time apparently was 5 ppm. Pre-employment and serial hematological measurements were obtained during the 3-year period and were compared with values from a control group. Approximately 40 percent of the workers exhibited mild hematological abnormalities during the first year, and this percentage declined greatly in subsequent years. The most common early sign of benzene hematotoxicity was mild thrombocytopenia (96 to 155,000/mm^3) followed by anemia, which was normochromic with a tendency toward subsequent development of hyperchromia (presumably because of an increase in mean corpuscular volume) after 1 year of employment. An initial increase in WBC count was followed in certain cases by leukopenia. In support of this observation Doskin cites Soviet literature describing a phasic response to benzene. Except for an early report by Hunter[91] suggesting increased erythropoiesis as an initial response to benzene, Western investigators have not described such a phasic response. Other somewhat different findings in Doskin's study include observations of lymphocytosis rather than lymphocytopenia and relatively greater effects in younger subjects, in contrast with Aksoy's finding of no effect of age on response to benzene.[3] Analysis of bone marrow of 30 workers showed hypercellularity, particularly in subjects with leukocytosis, a decrease of megakaryocytes in subjects with thrombocytopenia, and an increase in lymphocytes in younger subjects. In addition, a decrease in the phagocytic ability of leukocytes was noted. Although not substantiated, these findings suggest that exposure of workers to concentrations of 10 to 40 ppm benzene for less than 1 year produces mild hematological effects. Information about the benzene monitoring system would be of value in interpreting these findings.

Kozlova and Volkova[107] in the Soviet Union have also used the phagocytic function of leukocytes as an indicator of benzene hematotoxicity. They studied 252 workers exposed to benzene during a 5-year period in which benzene concentrations initially ranged from 47 to 310 ppm then, as control measures were improved, decreased to average concentrations of 25 to 47 ppm by the end of the period. The workers were classified in three groups depending upon exposure levels, the lowest being 24 to 39 ppm. All groups showed a decrease in cell counts, and severity of the changes was greatest with higher exposure levels. In the higher-level exposure group, the extent of the hematological effects correlated well with duration of exposure. The most prominent findings were leukopenia, predominantly reflecting neutropenia, and thrombocytopenia. Changes in red cells tended to occur relatively late. In most cases a decrease in phagocytic activity occurred earlier than other hematological effects.

Another study from Eastern Europe[100] reported that 16 of 27 workers experienced an increase in levels of red cell delta-aminolevulinic acid, a precursor in the heme biosynthetic pathway. In 12 of the 16, earlier benzene exposures are stated to have ranged from 6.4 to 15.6 ppm and more recent exposures were to 1.6 ppm. The remaining four workers were exposed only to 1.6 ppm benzene. Blood counts were normal. The pertinence of these findings must await confirmation of the authors' hypothesis concerning the effect of benzene on porphyrin metabolism.[99,100] Also of potential but unproven importance is the finding by

Smolik et al[168] of a decrease in the mean serum complement of 34 workers as compared to those of a control group. Benzene levels ranged from 3.4 to 6.8 ppm, and duration of exposure, from 3 months to 18 years. Other investigators also have suggested that benzene alters the immune system in man, including the frequent observation of lymphocytopenia and reports of altered serum immuno-globulin levels, the presence of antibodies against circulating blood cells, and morphologically altered lymphocytes and monocytes.[111,112,149,151] Based on the immune surveillance hypothesis, it could be conjectured that a decrease in immune function plays a role in benzene leukemogenesis. At present, however, the evidence does not clearly delineate a primary effect of benzene on immune function in man.

In a study performed in Korea, Chang evaluated hematological toxicity in relation to measured benzene levels and urinary phenol excretion in workers occupationally exposed to benzene.[41] The author presents data indicating hematological effects in workers exposed to concentrations as low as 20 ppm; by extrapolation he concludes that "benzene poisoning may occur when workers are exposed to as low as 10.1 ppm benzene in the air." Unfortunately, many details are missing in the published account of this study. After eliminating workers with various disease states from the study group, the author evaluated 119 individuals said to be exposed to benzene in an unspecified industrial area. Benzene levels were measured by a method based on ultraviolet absorption in ethanol, but the number and duration of measurements are not given. Urinary phenol was measured after 4 hours of work by reaction with p-nitroaniline. The number of such determinations for each worker is not specified. Of the 119 subjects, 28 showed hematological abnormalities: 21 with a normochromic or hyperchromic anemia, 2 with leukopenia, and 5 with both anemia and leukopenia. In comparison with other workers, those with hematotoxicity generally had been exposed for a shorter duration at higher benzene levels. Extrapolation from a plot of benzene concentration (in ppm) against interval from start of work until occurrence of hematotoxicity revealed an exponential function described as $y = (82.5)(0.77^{0.2x}) + 10.1$; where y is ppm benzene and x is work duration in months (Figure 2). No hematological toxicity was observed in the 18 subjects exposed to 10 to 20 ppm benzene. The graph suggests that 7 of the 24 subjects exposed to 20 to 30 ppm benzene exhibited hematological toxicity after 42 to 96 months of exposure. At the higher benzene levels apparently 1 of 5 workers exposed to 100 to 120 ppm benzene and 4 of 13 workers exposed to 100 to 110 ppm benzene developed hematological toxicity. Of interest is that the average duration of work for all subjects was substantially less at the higher benzene levels, perhaps implying an occupationally related attrition. Studies of phenol excretion showed a direct correlation with measured benzene levels ($r = 0.469$, $p < 0.01$). The levels of urinary phenol in this study were about 2 to 3 times lower than those reported by Elkins for equivalent benzene concentrations. The author suggests that this disparity may be due in part to his use of a 4-hour exposure period rather than the 8-hour exposure used by Elkins. The urinary phenol findings suggest that the author has not underestimated the atmospheric benzene concentration at the time of the study. This interesting study presents the most detailed evaluation of benzene hematotoxicity versus dose available in the literature. Unfortunately, because the information is incomplete, particularly as to characterization of the work force and exposures, it is difficult to interpret the relevance of these findings to benzene exposure in other populations.

Numerous cases of benzene hematotoxicity in Italy have been reported, particularly by Vigliani, Saita, Forni, and their colleagues.[58-62,155-162,189-194] The accumulated case reports in Milan and Pavia among shoe workers and other occupationally exposed groups have been reviewed recently[191,193] and are described below. Measured benzene concentrations have ranged from 25 to 1500 ppm and often have been over 200 ppm.

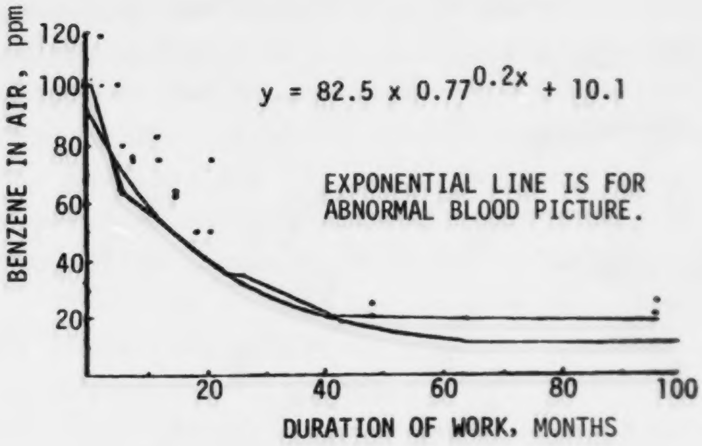

Figure 2. Effect of benzene in air and duration of work on abnormal blood picture.

Among the many French investigators who have reported benzene hematotoxicity, Girard and his colleagues present an extensive series of investigations.[67-75] Their studies include the frequent observation of a decreased leukocyte alkaline phosphatase in a group of 319 workers exposed to benzene levels said to range from 10 to 25 ppm.[67,68] They have also noted a statistically significant occurrence of a history of occupational benzene exposure in patients with aplastic anemia as compared to a control group.[72,73]

Studies of Aksoy et al

Aksoy and his colleagues in Turkey have performed one of the more extensive evaluations of individuals with benzene hematotoxicity.[3-15] Most of the cases observed were in shoe workers who between 1955 and 1960 began using an adhesive containing high levels of benzene. Individual cases of aplastic anemia were first noted in 1961. By 1977 Aksoy and his colleagues had studied 46 patients with this disorder, of whom 14 had died of aplastic anemia, 5 had developed acute leukemia, 1 developed myeloid metaplasia, 22 were in complete remission, 2 were still under treatment, and 2 were lost to follow-up.[16] This compilation includes only patients personally observed by Aksoy et al. Thirty-five were shoe workers and the remainder were also exposed to adhesives containing 9 to 88 percent benzene. Exposure was generally in small shops with poor ventilation. Where measured, the benzene concentrations ranged from 150 to 650 ppm.

Aksoy and his colleagues also present a thorough study of hematological findings in 217 apparently healthy male shoe workers in comparison with 100 control subjects.[3] Fifty-one of the 217 exposed were considered to have a benzene-associated hematological abnormality. Forty-one were afflicted with leukopenia and/or thrombocytopenia; the other ten are included on the basis of various abnormalities including eosinophilia, basophilia, giant platelets, lymphocytosis, and an acquired Pelger-Huet anomaly. The latter is a morphological abnormality seen in leukemia and reported in association with benzene exposure. The authors also report that 33 percent of the total work group was anemic, but because the hematocrit readings returned to normal following iron therapy in those treated, the anemia is not definitively ascribed to benzene toxicity. Macrocytosis was not observed, but the absence of this finding may be due to the complicating iron deficiency anemia, which usually leads to microcytosis. No difference in the relative incidence of benzene hematotoxicity was noted in different age groups. Of note was a tendency toward a higher incidence of hematotoxicity in workers exposed for less than 1 year as compared with those exposed for longer periods. The authors suggest that this could be due to all hematological changes occurring or starting in the first year of exposure, or to a loss of affected individuals from the work force.

Aksoy's group has also detailed the hematological findings in 32 patients with clinically significant pancytopenia.[5] All used adhesives containing benzene. Ambient levels of benzene were said to range from "15 and 30 ppm outside working hours and were recorded to reach 210 or, rarely, 640 ppm when benzene containing adhesives were being used." Cellularity of the bone marrow was decreased in 12 cases, normal in 12, and increased in 7. In the remaining case, giant erythroid precursors were observed, perhaps indicating preleukemia. The severity of disease and likelihood of fatal outcome was greatest in the group with hypocellular bone marrows, although the duration of exposure appeared unrelated to the bone marrow findings. Macrocytic erythrocytes were observed in 14 patients, most of those being patients with hyperplastic bone marrows. Nucleated erythrocytes were noted in the peripheral blood smear of six patients. Other red cell abnormalities included a mild to moderate increase in the osmotic fragility, with or without 24-hour preincubation, in 13 of 20 subjects tested; an increase in fetal hemoglobin in 20 of 24 patients; and a decrease in Hb A_2 in 3 of 24 patients.

This group has described additional studies suggesting an increase in hemoglobin F in benzene hematotoxicity.[4,9] Evidence of a shortened red cell life span included a slight reticulocytosis, mild hyperbilirubinemia, and increased urobilinogen in some subjects, and a moderately shortened radioactive chromium survival in the one subject in whom this was measured. Except for the latter finding, these observations could also be due to ineffective erythropoiesis. White cell abnormalities included an absolute lymphocytopenia in 24 subjects, a slight increase in monocytes in 4 subjects, and the development of a Pelger-Huet anomaly in 1 subject following recovery from pancytopenia. A low platelet count (thrombocytopenia) was observed in 28 individuals, and the peripheral blood smears showed giant or morphologically abnormal platelets.

Miscellaneous Abnormalities in Benzene-induced Pancytopenia

There is evidence that in addition to producing a decrease in number of circulating blood cells, benzene exposure causes formation of abnormal red cells, platelets, and white cells. The most common red cell alteration is macrocytosis, a condition that also occurs in erythroleukemia and in vitamin deficiency states associated with abnormal metabolism of nucleic acid. Megaloblastic and macroerythrocytic precursors have been noted in the bone marrow of patients with benzene toxicity.[44,47,81] Abnormal red cell function is also suggested by the finding of significant hemolysis in occasional cases of benzene hematotoxicity,[19,55,57,92,121,139,151,158] by a report of abnormal osmotic fragility in one[7] but not another[79] study, and by indirect indications of altered heme synthesis, including changes in levels of porphyrin in blood and urine and in levels of delta aminolevulinic acid in red cells.[99,100,167] A number of investigators report abnormal morphology and function of granulocytes. Observations include a decrease in phagocytic function,[107] a loss in leukocyte alkaline phosphatase,[67,68] an altered osmotic fragility,[143] a change in the fluorescence characteristics of leukocyte nuclei,[106] and a Pelger-Huet anomaly.[3,159,166,197] Many studies suggest that benzene hematotoxicity results in altered platelet function,[29,44,56,97,104,138,160,161,164,171] perhaps leading to an increased susceptibility to bleeding. Morphological abnormalities of circulating platelets and of megakaryocytes have also been reported.[3,161,170] No direct evidence of altered lymphocyte function is available, although this has been suggested on the basis of apparent alterations in immune function in benzene-exposed individuals.[111,112,149,150,168] At present, however, it is unclear whether the reported immunological findings are a primary effect of benzene, are secondary to the formation of altered hematopoietic cells, or reflect some unrelated situation. In addition, cytogenetic aberrations in circulating lymphocytes have been observed (see Section 1). Abnormal monocytes in the blood of workers exposed to benzene also have been reported.[153]

Many of these abnormalities in circulating red cells, white cells, and platelets are reported to occur as relatively early manifestations of benzene hematotoxicity. For this reason they have been proposed for use in screening tests to detect early benzene effects. An absolute lymphocytopenia is noted as a relatively early indication of benzene hematotoxicity by some[3,79] but not other[86,90] investigators. An increase in circulating eosinophils and basophils is also sometimes observed.[3,5,25,32,90,91] In addition, the serum levels of various enzymes[71,115] are reported to be altered in early benzene hematotoxicity. Further systematic study of possible indicators of benzene hematotoxicity would be of value in screening exposed individuals for the detection of early damage to bone marrow.

Although animal studies provide evidence of rapid interference in erythropoiesis following administration of benzene, the relatively long normal red-cell survival period of 120 days causes any diminution in red cell production to be manifest only slowly as a decrease in circulating red cells. In contrast, the normal period of platelet survival is 7 to 10 days, and survival of granulocytes in the circulation is perhaps 24 hours. Although a red cell count measures almost all mature red cells, about 30 percent of platelets are sequestered in the spleen, and the measurable circulating granulocytes represent less than 20 percent of the total in the body. As a further complication, there is a relatively large reserve of bone marrow, which is capable of perhaps a sixfold greater output of mature cells in a normal situation. Accordingly, counts of the circulating cells do not give an adequate index of very early benzene hematotoxicity.

Long-term Evaluations

The prognosis in mild cases of pancytopenia is good if benzene exposure is discontinued. In some individuals, however, pancytopenia has progressed even after presumed cessation of exposure, and apparent hematological recovery has sometimes been followed by acute leukemia occurring as late as 15 and 27 years after the initial findings.[49,98] Relatively few longitudinal assessments of occupational exposure groups have been performed. One such study was reported by Hernberg et al,[90] who reevaluated a group of individuals exposed to benzene in a shoe factory, originally studied by Savilahti.[163] Benzene had been in use for about 10 years, and levels close to 400 ppm were measured. The original investigation disclosed abnormal blood counts, most commonly thrombocytopenia, in 107 of 147 workers studied. Ten of these individuals required hospitalization, one of whom died with severe pancytopenia. Of note is that one of the more severely affected individuals, whose peripheral blood counts returned to normal in 2 years, subsequently developed acute leukemia. Hernberg et al restudied 125 of the original workers in comparison with a nonexposed control group 9 years after cessation of benzene exposure. There was definite improvement in mean platelet count, which increased from 176,480 to 257,360. However, the platelet count in the group exposed to benzene remained significantly ($p<0.01$) lower than that of the control level of 293,000 per mm^3. The mean erythrocyte count for male workers was stable during this 9-year period and was also significantly lower than that of the control group (4.4 vs 4.7 million per mm^3; $p<0.01$). Improvement to control levels was observed in the mean erythrocyte count of female workers and the mean leukocyte count of the entire group exposed to benzene. These findings suggest that recovery after benzene hematotoxicity may occur very slowly or may be incomplete. Of interest is the observation that the reported mean platelet and red cell counts in the exposed population were within normal limits, and the observation of a persistent benzene effect in this population required simultaneous study of a normal control group. A possible inference from this finding is that peripheral blood counts within the wide range of normal do not preclude an effect of benzene on hematopoietic tissue. A further observation by Hernberg et al[90] is that the severity of the original findings in 1955 did not correlate with the extent of recovery in 1964. This analysis, however, was not based on the blood counts of individual workers but rather on a comparison of the initially severe with the initially mild cases.

Another long-term follow-up of an occupationally exposed group has been reported by Guberan and Kocher.[84] Their evaluation of 216 of 282 workers 10 years after apparent cessation of benzene exposure revealed two individuals with isolated thrombocytopenia, one with anemia and thrombocytopenia, and one with mild pancytopenia. They note that one worker had died of aplastic anemia 9 years after cessation of benzene exposure. Few additional details are provided. Further study of the long-term consequence of occupational exposure to benzene in well-defined cohorts would be of great interest.

LEUKEMIA

Leukemia can be defined as a neoplastic proliferation and accumulation of white blood cells in blood and/or bone marrow. Hematologists generally agree concerning characterization of the four main types of leukemia: acute and chronic myelogenous (also known as granulocytic) leukemia, and acute and chronic lymphocytic leukemia. This classification represents diseases that differ in terms of incidence, course, prognosis, and, presumably, etiologic factors. In addition, there are other types of leukemia, related to these four major types, about which there is some disagreement concerning diagnostic criteria. Erythroleukemia, acute promyelocytic leukemia, stem cell leukemia, and acute myelomonocytic leukemia, all of which have been reported in association with benzene exposure, are generally considered to be variants of acute myelogenous leukemia. There is, however, some question as to whether the relatively rare acute monocytic leukemia (Schilling type) is an entity of its own or is related to acute myeloblastic leukemia.

Chronic myelocytic leukemia has often been classified under the heading of myeloproliferative syndrome, which includes polycythemia vera, myelofibrosis and myeloid metaplasia, and essential thrombocythemia. These disorders have in common a neoplastic proliferation of the pluripotential stem cell responsible for the formation of granulocytes, platelets, red blood cells, and perhaps, fibroblasts and other monocytic cell types. They also share a potential for the development of acute myeloblastic leukemia. Although the myeloproliferative disorders may overlap clinically, chronic myelogenous leukemia does appear to be distinct and readily separable on the basis of a characteristic chromosomal abnormality and other distinguishing features. The cells identifiable in the blastic crises of chronic myelogenous leukemia may be more closely related to lymphoblasts than to myeloblasts, again consistent with the pluripotential nature of the stem cell discussed above.

Investigators have associated exposure to benzene with preleukemia. Preleukemia is very difficult to define, and there is little agreement as to clinical criteria. In most cases, the diagnosis is made in retrospect after the patient has developed a clearcut acute leukemia, usually of the myeloblastic type. A characteristic case history would be a benzene-exposed individual with pancytopenia whose bone marrow demonstrates generalized hypoplasia, but with a slight increase in somewhat atypical blast cells. Over a period of weeks to years, repetitive bone marrow measurements show a gradual increase in these blast forms until frank acute myeloblastic leukemia is indicated by almost complete blastic replacement of the bone marrow and by the presence of these cells in the peripheral blood. Preleukemia is defined as beginning at the point at which there is a reasonable expectation that the patient would develop acute leukemia and ending at the point at which acute leukemia is diagnosed. Despite much current interest in the subject, no criteria are agreed upon to define these two points. Accordingly, it is difficult to evaluate literature concerning preleukemia in benzene-exposed individuals beyond indicating that such a continuum is often reported.[6,10,72,117,175]

Relationship of Benzene to Leukemia

The evidence concerning the relationship of benzene and acute leukemia has been reviewed by a number of authors and panels in recent years. Most of these individuals and groups accept causative role for benzene in human acute myelogenous leukemia and it is so described in the routine hematological literature. The following quotations are from three standard American textbooks on hematology:

> "A variety of chemicals and drugs have been suggested as possible leukemogenic agents in human leukemia, but only benzol can be unequivocally implicated."[42]

> "Any chemical capable of producing myelotoxicity must be regarded as a potential leukemogen, if the findings in radiation-induced leukemia apply which indicate that cell damage with depression of marrow function may produce alterations leading to the transformation of damaged cells into neoplastic ones. The only chemical which has been clearly identified as one which increases the incidence of myeloid leukemias in man is benzene in rather heavy occupational exposure. The development of stem cell, erythroblastic and myeloblastic leukemias and persistent chromosome abnormalities in exposed individuals known to have had neutropenia is especially significant."[154]

> "Sporadic cases of acute leukemia have occurred after exposure to chemical agents such as benzene, phenylbutazone, and chloramphenicol. Aplastic anemia and acute myelogenous leukemia have followed prolonged exposure to benzene. About 15% of the patients who develop hematologic abnormalities due to benzene exposure also develop acute leukemia. Leukemia is usually preceded by a period of bone marrow aplasia."[33]

Other assessments of this relationship, however, leave some room for doubt. Thus, a recent document from the National Cancer Institute[109] concluded:

> "At best _benzene_ must be _considered_ as a _suspect_ leukemogen."

In evaluation of the causal relationship of benzene to acute leukemia, three types of information are pertinent: the nonleukemic effects of benzene, case reports of benzene-associated leukemia, and results of epidemiological studies.

Nonleukemic Biomedical Effects of Benzene--

Benzene clearly damages hematopoietic tissue in man. That this damage can be due to benzene alone is supported by observation of pancytopenia in diverse exposures in many different countries, as well as by animal studies in which administration of benzene in the absence of any other solvent produces pancytopenia (see Section 3). A somewhat negative point is the absence of conclusive evidence of leukemogenic action of benzene in animals. Arsenic may be the only other compound for which there is good epidemiological evidence of carcinogenesis in man in the absence of an analogous animal model.

The fact that patients with idiopathic aplastic anemia or aplastic anemia developing from other agents, most notably chloramphenicol and phenylbutazone, may also progress to acute leukemia[23,133] provides inferential evidence that benzene-induced damage to hematopoietic stem cells could lead to acute leukemia. The mutagenic effects of benzene, particularly in relation to chromosomal abnormalities in man, may also be considered inferential evidence supporting a causal role of benzene in leukemia (see Section 2).

Case Reports of Leukemia Associated with Benzene--

Well over 100 cases of leukemia in benzene-exposed individuals have been described in the literature since the original report in 1928. However, enumeration of case reports does not, by itself, provide definitive evidence that benzene is a causative factor in acute leukemia. There is a finite probability that each case represents a chance association with benzene exposure in an individual whose leukemia is due to some other cause. Reports of single cases and small series of cases generally lack information concerning the size of the population at risk that is required for firm conclusions. On the other hand, a number of factors would tend to lead to an underreporting of benzene-associated leukemia. The general acceptance of this relationship in the medical literature for some time would tend to hinder the preparation or publication of manuscripts describing one further case. A long period often follows benzene exposure before the onset of acute leukemia; this delay could lead to the relationship being overlooked by the patient or physician.[6,49,83,98,116,150,162,166,193] Furthermore, because benzene is a ubiquitous component of our chemical age, exposure may be unrecognized. Accordingly, simple quantitation of case reports in the medical literature cannot provide definitive information concerning the causal relationship of benzene to acute leukemia.

These case reports, however, do provide presumptive evidence of a leukemogenic effect of benzene. Of particular note is the frequent description of persons suffering from benzene-associated pancytopenia in whom evolution to acute leukemia was observed. Idiopathic aplastic anemia is an uncommon disorder, reported far less frequently than acute myelogenous leukemia. The relatively frequent documentation of benzene-associated pancytopenia progressing to acute leukemia, which is in keeping with that observed in other causes of aplastic anemia, further supports the possibility that exposure to benzene increases the risk of developing acute leukemia. Similarly, the case reports are also pertinent for the frequency with which evolution of the hematological findings progress to or through an erythroleukemic stage.[10,20,51,52,59,63,105,110,139,175] The relative occurrence of the erythroleukemia variant in these case reports appears to be more common than that observed in cases of acute myeloblastic leukemia and its variants in the general population. Unless this represents some unexpected bias in the reporting process, the frequency with which erythroleukemia is observed in benzene-exposed patients also supports a specific leukemogenic action of benzene.

Epidemiological Studies--

Evidence supporting a role for benzene in leukemia has been obtained in a number of epidemiological studies. Three general approaches have been used. First, the discovery of a relatively large number of individuals with leukemia having a history of occupational benzene exposure has led to assessment of the likelihood of this occurrence. This has been done by estimating the number of workers in the occupational group and computing the incidence of leukemia in comparison with that expected in the general population. A second approach has been to obtain occupational histories of persons with leukemia and compare the incidence of potential benzene exposure in these leukemics with that of control populations. The third approach has been to evaluate the mortality characteristics, including leukemia deaths, of relatively large populations working in an industry involving known benzene exposure. Any high incidence of leukemia can be further analyzed in terms of occupational subgroup, duration of employment, and other factors.

Each of these approaches entails advantages and disadvantages. For example, the third approach, representing a standard epidemiological technique, is by far the most thorough in its characterization of an entire work force; however, the validity of information on leukemia incidence depends on the vagaries of death certifications rather than on direct observation. In contrast, enumeration of the population at risk in the first approach represents a crude estimate, and little is known about the population characteristics of the occupational group. This approach does offer a high level of confidence in the diagnosis of leukemia, in that the subjects have been observed by the investigators.

The relatively recent studies of Aksoy and his colleagues in Turkey strongly support the causal relationship of benzene exposure to acute leukemia.[6,10,11,13,14,16] Their evidence includes individual case reports of workers with aplastic anemia which progresses through a preleukemic phase to frank acute myeloblastic leukemia or erythroleukemia; an accumulation of cases resulting in a statistically significant higher incidence of acute leukemia among shoeworkers; and an outbreak of leukemia in this population that appears temporally related to the onset of benzene use and that has subsided following replacement of benzene as a solvent for adhesives. From 1967 to 1973 Aksoy et al observed 26 patients with acute leukemia among shoe workers.[10,11,16] Analysis in selected work areas revealed that "the concentration of benzene was found to reach a maximum of 210-650 parts per million when adhesives containing benzene were in use." There were 14 cases of acute myeloblastic leukemia, 4 of preleukemia, 3 of acute erythroleukemia, 3 of acute lymphoblastic leukemia, and 1 each of acute promyelocytic and acute monocytic leukemia. Duration of benzene exposure ranged from 1 to 15 years. The authors state that there were 28,500 shoe workers at risk in Istanbul and derived a leukemia incidence of 13 per 100,000 in this population. This incidence is statistically significantly higher ($p<0.02$) than the risk of 6 per 100,000 assumed for the general population. Because this latter number apparently is derived from the incidence of leukemia in developed nations, rather than being specific to Istanbul, there is some degree of uncertainty. This appears to be more than counterbalanced, however, by a number of factors. Foremost is that the distribution of cases reported by Aksoy et al strongly differs from that of leukemia in the general population. If the relative incidence were computed solely for acute myeloblastic leukemia and its variants, a magnification of the risk in benzene-exposed shoe workers would be observed. Secondly, Aksoy et al apparently have not age-adjusted their findings. In their series, the average age at the time of diagnosis was 34.2 years. This is a relatively low-risk age period for leukemia, with a reported death rate about half of the overall incidence.[43] Recalculation of their data with an age factor would presumably increase the statistical significance of the findings. In addition, Aksoy has recently stated that his studies underestimate the relative risk of leukemia because the incidence of leukemia in the general population of Turkey is 2.5 to 3.0 per 100,000 and because shoeworkers with acute leukemia probably were admitted to other Istanbul hospitals without his knowledge.[16]

Recently, Aksoy presented his observations of acute leukemia in shoe workers during the period 1967 to 1976.[16] As shown in Table 1, the peak incidence of leukemia in shoe workers occurred between 1971 and 1973. This follows by a few years the appearance of a notable incidence of aplastic anemia in this occupational group. The decline in cases since 1973 is temporally related to a decrease in use of benzene as an adhesive solvent, which began gradually in 1969. Aksoy also reports that pancytopenia was present in 27.5 percent of the cases before the onset of acute leukemia, which occurred 6 months to 6 years later. The hematological findings often indicated a period of recovery before the onset of leukemia, a phenomenon also noted by other investigators. Aksoy states that during this period, over 100 cases of aplastic anemia were observed that were either idiopathic or associated with an agent other than benzene, and in none of these cases did acute leukemia develop. He also states the opinion that no blood dyscrasia is required before the onset of leukemia and provides an example of a 23-year-old shoeworker who was hematologically normal when studied 4 years before the onset of acute erythroleukemia. As with other cases of leukemia associated with benzene exposure in which the patient had no detectable pancytopenia beforehand, the interval between hematological observations is too long to ensure that pancytopenia did not in fact occur.

A relationship between benzene exposure and leukemia is stressed in a series of reports by Vigliani and his colleagues in Northern Italy.[190,193] In recent review articles, Vigliani and Vigliani and Forni summarized their experience from 1942 to

Table 1. ANNUAL NUMBER OF LEUKEMIA CASES [a] AMONG SHOEWORKERS WITH CHRONIC EXPOSURE TO BENZENE IN ISTANBUL BETWEEN 1967 AND 1975[16]

Years	Number of leukemic shoeworkers	Years	Number of leukemic shoeworkers
1967	1	1972	5
1968	1	1973	7
1969	3	1974	4
1970	4	1975	3
1971	6	1976	0

[a] Four other leukemic workers with different jobs and two leukemic individuals outside of Istanbul are not included in this series.

1975.[189,190] They observed 66 cases of significant benzene hematotoxicity in Milan, of which 11 were cases of acute myeloblastic leukemia or its variants. All 11 leukemia patients died, and 7 subjects died of aplastic anemia. In Pavia between 1959 and 1974 there were 135 patients with benzene hematotoxicity; 13 died of acute myeloblastic leukemia or its variants, and three of aplastic anemia. All of the patients in Pavia were workers in the shoe industry. Measured benzene concentrations in the breathing zone of workers using glue usually were 200 to 500 ppm, and ranged from 25 to 600 ppm. Many factory shoe workers also worked at home, where presumably further benzene exposure occurred. Shoe workers were also the largest single occupational group with hematotoxicity in Milan, although many other working groups were also affected. Of interest is an apparent outbreak of benzene hematotoxicity, including eight cases of severe pancytopenia and two cases of myelogenous leukemia, in the rotogravure industry. This outbreak was temporally related to the use of inks and solvents containing large amounts of benzene. Ambient benzene levels were calculated to be between 200 and 400 ppm, with peaks up to 1500 ppm. In an earlier study, Vigliani and Saita[193] estimated the number of workers exposed to benzene in Pavia and Milan and, based on the incidence of acute leukemia in the general population of Milan, calculated a 20-fold higher risk of acute leukemia in these workers.

An interaction between benzene and radiation in leukemogenesis is suggested by the study of Ishimaru et al.[95] These authors performed a retrospective study of survivors of the two atomic bombings in Japan, evaluating the effect of occupation on the incidence of leukemia. Controls were matched with patients by age, sex, residence, and distance from the atom bomb explosion. The occupational history of leukemic patients was obtained from relatives. Of 492 cases of leukemia through 1967 in this population, adequate histories and appropriate controls were obtained for 413, of whom 303 were adults. Comparison of the controls and leukemic patients revealed that the risk of leukemia was 2.5 times higher in those with an occupational history potentially related to benzene or X-rays. The risk was significantly higher in those with 5 or more years of potential exposure but not in those who had been employed in such occupations for less than 5 years. The relative risks were similar in Hiroshima and Nagasaki and were higher for acute leukemia (2.9) than for chronic leukemia (1.8). The major limitation of this study is the possible inaccuracy of the occupational history of leukemic subjects as compared with that of the living controls.

Girard et al[72,73] have evaluated the frequency of a positive history of benzene exposure in 401 hospitalized patients with serious hematological disorders as compared with 124 patients hospitalized for nonhematological problems. A statistically significant increase ($p < 0.05$; $\chi^2 > 3.84$) in history of benzene exposure was noted for patients with aplastic anemia (10 of 48, $\chi^2 = 12.2$), acute leukemia (17 of 140; $\chi^2 = 5.6$), and chronic lymphocytic leukemia (9 of 61; $\chi^2 = 6.7$) as compared with the control group (5 of 124).

The rubber industry has long been characterized by exposure to various solvents including benzene. Pancytopenia associated with benzene exposure has been noted in workers in a number of countries (see above). In the United States, Wilson[195] reported a large cohort of individuals exposed to benzene during the expansion of rubber production early in World War II. Some degree of cytopenia was noted in 83 of 1104 individuals studied. Leukemia was not reported. Ascertaining the present vital status and causes of death in this cohort, particularly in relation to leukemia incidence, could be of great value.

Other studies of workers in the rubber industry include a report of the U.S. Department of Health, Education, and Welfare.[185] This report cites a 54 percent increase in the death rate for cancers of the lymphatic and hematopoietic system in male rubber-industry workers dying in 1950 as compared with workers in all manufacturing industries. There were also a 30 percent increase in death rate due to large bowel cancer, a 19 percent increase due to cancers of the respiratory tract, and an 8 percent higher overall cancer death rate.

Mancuso and his colleagues[119,120] performed a series of studies of occupational cancer rates, with particular emphasis on the rubber industry, including evaluation of a cohort of 1977 workers. No apparent increase in hematopoietic cancers was observed. There was, however, a higher incidence of tumors of the gallbladder, bile duct, and salivary gland in occupational subgroups not associated with high solvent exposure.

A comprehensive series of studies[17,18,126-129,182] of the health status of rubber-industry workers has been performed by the Occupational Health Studies Group of the University of North Carolina. They have evaluated the 10-year mortality experience of a large cohort of male workers (5106 deaths) at four tire manufacturing plants. The subjects were in the work force or were retired in 1964. This series of studies was reviewed recently by Troler.[182] The mortality due to all cancers (1014) was normal or slightly elevated, depending on the data base used for comparison. Deaths due to cancer of the lymphatic and hematopoietic system (total of 109) were 31 percent higher than expected and were increased in cohorts of each of the four companies. In the category of lymphosarcoma and Hodgkin's disease, the standard mortality ratio (SMR) was 129 and an increase in the expected number of deaths was observed in two of the four company cohorts. Similarly, for deaths due to all forms of leukemia the SMR was 130 and the increase was observed in three of the four cohorts. When this latter category was further subdivided, the overall SMR for lymphatic leukemia was found to be 158 and the expected death rate was elevated in two of the four company cohorts. Of particular note is that the SMR for deaths attributed to lymphatic leukemia was 291 in the age group 40 to 64.

Several approaches were followed in further study of the increased incidence of lymphatic leukemia. Contrasting the work history of 17 patients with lymphatic leukemia with those of three matched controls for each case revealed that solvent exposure increased the overall risk by a factor of 3.25. Further classifying the groups according to high, low, and medium solvent exposure, yielded a 5.5 factor for the high-exposure group. In those patients first subjected to high exposure between 1940 and 1960, the factor for the relative risk of lymphatic leukemia was 9.0. The relationship of solvent exposure to lymphatic leukemia was statistically significant at $p < 0.025$. The study also showed an increase in the mean difference in years of work history between lymphatic leukemia and the case controls. This was inversely proportional to the extent of solvent exposure. Tyroler has cited several epidemiologic reasons why these findings must be interpreted with caution.[182] These include the dependence on death certificates for diagnostic information, which apparently is now being validated with clinical and pathological findings. Tyroler notes good agreement between the death certificate and medical information concerning deaths stated to be due to leukemia. It would be pertinent to learn whether the deaths certified as caused by lymphatic leukemia represent cases of chronic lymphatic leukemia, a relatively common disorder in this age group, or of acute lymphoblastic leukemia. The latter is rare in adults and is sometimes difficult to distinguish from the acute myeloblastic and stem cell leukemias that are classically associated with benzene exposure. Other conceivable limitations to the studies by the University of North Carolina group include the possibility that the association between work history and nonfatal cases in this cohort might be systematically different from that observed in fatal cases, and that individuals with leukemia and a work history of solvent exposure may also have had uncontrolled exposures to radiation, drugs, or other environmental agents that might cause the observed increase in leukemia rates. A further limitation is the lack of historical data concerning the benzene exposure or the concentrations of other solvents. These studies do, however, strongly support the possibility that long-term exposure to benzene in the U.S. rubber industry leads to an increased risk of lymphatic leukemia.

Monson and Nakano also have recently studied mortality of workers in the rubber industry.[136,137] Evaluation of a cohort of 13,571 white males in one plant in Akron, Ohio, revealed an SMR of 82 for all causes of death and an SMR of 94 for all malignant neoplasms in comparison with U.S. mortality statistics.[136] When the causes of death were further subdivided by types of neoplasm, the highest SMR (128) was observed for leukemia (43 expected, 55 observed). This high incidence was observed particularly in workers in the tire (SMR 150) and processing (SMR 240) divisions, but was also observed in other worker subgroups. The excess in leukemia was notable in those who were less than 25 years of age at the time of employment and in those who began work before 1935. However, reevaluating the data for the 7374 employees who had worked at least 25 years gave no evidence of a decrease in risk for those who began working after 1935. The SMR for non-leukemic hematological neoplasms (lymphatic tumors and myeloma) was 101 in the overall cohort and 160 in the workers employed in the tire building area. Studies were also performed in three additional cohorts from this plant; female workers, black male workers, and salaried white males.[137] Data on these groups showed a tendency toward an increase in deaths due to lymphatic tumors and myeloma (SMR's 111, 125, and 115, respectively). Deaths due to leukemia were not greater than expected, although generally higher than the SMR for all causes of death. Evaluation of 574 deaths in white males working in other factory locations at the same rubber company revealed 14 deaths due to all lymphatic and hematopoietic tumors as opposed to 11.8 expected.

Other epidemiological studies of individuals potentially exposed to benzene have failed to reveal an increased incidence of acute leukemia. Thorpe reported an extensive study of leukemia mortality rates for the period 1962-71 in 36,000 employees and annuitants of eight European affiliates of a major petroleum company.[178] The report is noteworthy for the extensive discussion of methodological problems inherent in such retrospective studies. These problems include the low incidence of leukemia in the general population; the general inability to quantitatively define benzene exposure in the selected population; the difficulty of obtaining both accurate occupational histories and complete follow-up of the workers; and the problems of verifying the diagnoses of leukemia and of the subtype of leukemia.

In Thorpe's study four of the affiliates reported 18 cases of leukemia, whereas the other four reported none. In only 6 of the 18 cases was the subtype of leukemia known. Benzene exposure was believed to have occurred in 8 of the 18 individuals although the author points out the difficulty of clearly distinguishing among the job categories relative to benzene exposure. The data revealed no statistically significant increase in the leukemia rate over that expected in the population. There was, however, a tendency toward higher leukemia rate in the benzene-exposed as compared to the unexposed work groups. This study has been criticized by Brown[38] and defended by Thorpe.[177] The major criticism concerns factors possibly leading to an underreporting of leukemia incidence in this population.

Although benzene is associated with coke oven operations, study of cancer mortality in coke plant workers has revealed n statistically significant increase in leukemia incidence.[146]

This is also true for coke by-product workers, the occupational subgroup expected to be subjected to benzene exposure. These workers, however, constitute a relatively small group. Redmond et al[147] studied 345 individuals working 5 or more years in a coke by-product area. They found only 10 fatal neoplasms, of which 2 were of the lymph and hematopoietic tissue category (1.1 expected). Accordingly, no firm conclusions can be reached concerning benzene leukemogenesis in coke oven workers.

A risk of benzene exposure might also be expected in other occupations that are not well characterized epidemiologically. Adelstein examined the standardized proportional mortality ratios for various cancers in British males, 1959-1963, listing them according to 27 occupational groups.[1] The highest level of leukemia deaths was in the group described as "professional, technical workers, artists" ($p < 0.01$). This presumably would include chemistry professors, laboratory technicians, and other professional groups with relatively frequent exposure to benzene. Artists may be particularly at risk because they use unknown solvent mixtures in poorly ventilated and unregulated work areas. As with other studies, however, an exposure to benzene cannot be defined and it is possible that other factors, such as radiation, may be responsible for the increased incidence of leukemia in this occupational group.

In the United States, Li et al[113] evaluated the cause of death of 3637 members of the American Chemical Society who died between 1948 and 1967. Among deaths occurring between ages 20 and 64, an increase in all malignant neoplasms was noted (444 observed, 354 expected, $p < 0.001$) as compared with a control group derived from U.S. professional men. The increase in tumors of the lymphatic and hematopoietic system (94 observed, 50 expected) was the most highly statistically significant ($p < 0.001$) of all tumor types. Further subdivision revealed that the major increase was in nonleukemic hematopoietic and lymphatic tumors (61 observed, 34 expected, $p < 0.001$). The difference in number of leukemia cases (33 observed, 25 expected) was not statistically significant. Among deaths occurring after age 64, in comparison with U.S. white males, the highest level of statistical significance again was observed for all tumors of the lymphatic and hematopoietic system ($p < 0.001$). In this analysis, the increased incidence of both leukemic (16 observed, 9 expected) and nonleukemic (17 observed, 9 expected) hematological neoplasms was statistically significant.

A recent and relatively thorough epidemiological evaluation of benzene-exposed workers was conducted by Infante et al of NIOSH.[94] These investigators evaluated the mortality of 748 white male workers exposed to benzene in manufacture of a rubber product. The cohort consisted of those employed at any time during the period 1940 through 1949, and the period of risk for death was January 1, 1950, through June 30, 1975. The two control groups were the general population of U.S. white males and a group of 1447 white males employed in the same state and time period for at least 5 years in manufacture of a fibrous glass product. The vital status of this second control group was determined as of June 1, 1972. In the benzene-exposed group, data concerning the vital status as of June 30, 1975, were available for approximately 75 percent of the workers. The remaining 25 percent were assumed to be living. This assumption leads to an underestimation of the determined risk of death due to leukemia or other causes within this group and presumably accounts for the observation of only 140 deaths as opposed to 187 expected. Among the 140 deaths, 9 were due to all lymphatic and hematopoietic cancer as opposed to 3.45 based on expectations for U.S. white males ($p < 0.05$) and 5.10 expected in the fibrous glass industry control group (not statistically significant). Of particular pertinence is that 7 of the 9 deaths in the benzene-exposed group were of leukemia, as opposed to 1.38 and 1.48 expected on the basis of the respective control groups ($p < 0.02$).

Four of the leukemias were reported as acute myelocytic, two as monocytic, and one as chronic myelocytic. The latter case was unusual in that the patient's age at death was 29 years, relatively young for chronic myelocytic leukemia, and there was only a 2-year period between initial exposure and death. In the other six individuals, this period ranged from 10 to 21 years. As pointed out above, the description of "monocytic" leukemia in two cases most likely represents the myelomonocytic variant of acute myelocytic leukemia, but this is uncertain in the absence of review of the clinical material. Infante et al also cite an eighth case who was not part of the cohort, having started employment in 1950. This person died at age 28 of myelocytic leukemia 3 years after the initial exposure.

The authors also use their data to determine the relative risk for myeloid and monocytic leukemias, as opposed to total leukemia, and conclude that the population studied had a tenfold increased risk of death from these forms of leukemia. This factor could be an overestimate due to lack of clinical confirmation of the cause of death. Furthermore, the authors state that the death data were recalculated on the basis of a 50.37 percent incidence of myelomonocytic types of leukemia in this age group. Because chronic lymphatic leukemia is common in this age group and is a relatively mild disease in which death commonly occurs from some other cause, the percentage of deaths caused by myelomonocytic types of leukemia may well be much higher than the 50.37 percent value used in the analysis. Counterbalancing this is the potential underestimation due to the assumption that 25 percent of the cohort, for whom vital status is unavailable, is living. The authors also discuss the presumed dose, which is described below. In general, this epidemiological study provides excellent confirmatory evidence of the causal relationship of benzene exposure to acute myelocytic leukemia.

Another recent epidemiological study is that of Ott et al* who report the mortality experience of 594 individuals occupationally exposed to benzene in the chemical industry. The study is notable for its stratification of the work force in terms of benzene exposure levels and for relatively detailed descriptions of the individual cases with hematological findings. The cause-specific mortality rates for the 102 individuals identified as deceased are said to agree well with those observed in a cohort study of over 8000 employees in the same location. No association with the benzene exposure dose was observed. Of note are two deaths ascribed to anemia (one aplastic anemia, one pernicious anemia), two deaths due to acute myelogenous leukemia, and one death ascribed to bronchopneumonia with myeloblastic leukemia listed as a significant other condition. The time-weighted average benzene exposure of the three individuals with leukemia was characterized as low (2 to 9 ppm) or very low (< 2 ppm). The expected incidence of myelocytic leukemia in the study population is said to be 0.8. Observation of three cases is of borderline statistical significance. Considering the relatively small number of deaths evaluated, it is not surprising that the findings are inconclusive. Future reanalysis of this well-characterized cohort would be of interest.

Relationship of Benzene Exposure Level to Leukemogenesis

The medical literature provides little information concerning the dose of benzene inhaled by individuals who subsequently developed acute leukemia. In those few reports where benzene levels are cited, the duration of monitoring has been clearly inadequate for estimation of individual exposure levels. This is particularly true for assessment of the leukemogenic effects of benzene as opposed to its effects in pancytopenia. Concerning the latter, there are a number of studies in which a large percentage of the work force has developed benzene hematotoxicity. In these instances the available monitoring information might reasonably be used to estimate the average exposure. In contrast, because leukemia occurs in only a very small percentage of the work force exposed to benzene, it is difficult to be certain whether the occasional individual who does develop leukemia might have undergone some high exposure, perhaps due to a specific job or to faulty work habits, that is not typified by an area-wide benzene monitoring system.

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*Ott, M.G., C. Townsend, W.A. Fishbeck, R.A. Langner. Mortality Among Individuals Occupationally Exposed to Benzene. Exhibit 154, OSHA Benzene Hearings, July 19 - August 10, 1977.

In the few studies of acute leukemia where benzene levels are reported, the concentrations generally have been above 100 ppm. These include the studies of Aksoy et al and Vigliani et al discussed earlier. In other reports, benzene levels associated with acute leukemia have been 220 ppm[103] and 63 to 517 ppm.[166] There is some indication that the duration of benzene exposure is longer for acute myelogenous leukemia than for pancytopenia. A recent tabulation showed far more cases of pancytopenia[3,5,7,101] than of acute myelogenous leukemia developing after less than 2 years of exposure. This interpretation is complicated by the apparent lag period between cessation of benzene exposure and the development of acute leukemia, an interval reported to be as long as 15[49] and 27 years.[98] Accordingly, the apparently longer period of benzene exposure in cases of acute myelogenous leukemia than in pancytopenia may not be a function of dose but rather of the biological lag period between initiation of leukemogenesis and its eventual clinical appearance. For instance, the two case reports of leukemia tabulated by Goldstein[78] as having less than 2 years exposure to benzene were those of Sellyei and Kelemen,[166] in which an 18-month exposure led to pancytopenia, development of a Pelger-Huet anomaly, and, 7 years later, acute myelogenous leukemia. Similarly, the case reported by Kinoshita et al[103] had an onset as aplastic anemia after 6 months of benzene exposure, with subsequent development of acute leukemia. Also perhaps pertinent are the findings of Aksoy et al in Turkey. In 1972, these authors reported 32 cases of benzene-associated pancytopenia and only 4 cases of acute myelogenous leukemia.[5,6] In 1976 they reported 20 cases of acute myelogenous leukemia or erythroleukemia,[10] whereas the total of pancytopenia cases had increased only to 46 by mid-1977.[16] Of the 44 patients with pancytopenia who were available for follow-up, 5 had subsequently developed leukemia.[16] Accordingly, it is not certain whether the generally longer exposure period associated with leukemia as opposed to pancytopenia is a reflection of benzene dose.

The study of Infante et al[94] provides the most information about dose. As described in detail above, in a determination of vital status in 1975, these authors noted a statistically significant increased incidence of leukemia (total of 7) in a cohort of workers identified as having been employed any time between 1940 and 1949 in a factory where benzene was used. The exhaust ventilation was said to be excellent. Data concerning benzene levels include a report in 1946 stating that "Tests were made with benzol detectors and the results indicate that concentrations have been reduced to a safe level and in most instances range from 0 to 10 or 15 parts per million" (emphasis added). It is also stated that 112 surveys conducted between 1963 and 1974 "indicated that employees' benzene exposure was generally below the recommended concentration in effect at the time of each survey" (emphasis added). This frequency of survey is less than once per month in the stated time period, and thus represents grossly inadequate information for determining the benzene exposure levels of the seven individuals who developed leukemia. These individuals may in fact have been exposed to concentrations of benzene well below the acceptable industrial hygiene limits, which ranged from 100 ppm maximum allowable concentration in 1941 to 10 ppm as a time-weighted average in 1971. It is also possible that these individuals were exposed to concentrations well above the permissible concentrations, which were not detected in the relatively infrequent monitoring surveys. This possibility is supported by recent testimony of Harris,[87] who cited a survey performed in 1973-74 of the pliofilm manufacturing plant studied by Infante et al, with the following observation:

> "The spreader and drying units are entered on an intermittent basis to remove damaged film and rethread rollers and to repair mechanical failures. It was reported to the survey team that workers can spend up to 30 minutes inside these units. Periodic inspections of shorter duration are also conducted inside the spreader. Samples inside these units indicate a highly dangerous level of benzene, ranging from 200 to 350 ppm. Chemical cartridge respirators are required but are often not worn."

Short-term exposure levels of up to 30 ppm benzene were also noted in other areas of this plant.

In summary, the available literature concerning the benzene levels associated with the development of acute leukemia is inadequate for generation of dose-response curves or for an analysis of risk related to dose.

Possible Mechanisms of Leukemogenesis

The mechanisms by which benzene produces leukemia are unknown. Of particular interest is whether leukemogenesis is independent of the pancytopenic effect of benzene or is directly or indirectly related to overt damage to stem cells. The former possibility would put benzene into the category of other carcinogens for which the regulatory approach is to assume that no safe level exists. Alternatively, if benzene leukemogenesis requires preexisting damage to hematopoietic tissue, perhaps associated with an error in repair, then it is possible that a true threshold for leukemia could be determined. The prerequisite for bone marrow toxicity might be sufficient damage to cause an overt decrease in circulating blood cells. If this is true, then the public could be protected against leukemia by a standard that precludes such overt toxicity. Furthermore, this would allow extrapolation, with suitable care, from animal inhalation dose-response studies of benzene hematotoxicity.

It is also possible, however, that leukemia results from bone marrow damage that is too slight to produce clinically recognizable cytopenias. This would not necessarily preclude use of an animal model if the parameters of bone marrow toxicity are sensitive enough. Both Vigliani[190] and Aksoy[11,16] describe patients with apparent benzene-associated acute leukemia in whom prior study revealed normal blood counts. The frequency of such blood counts, however, was not sufficient to rule out an undetected pancytopenic phase. Furthermore, it is conceivable that these were the individuals in the population whose leukemia was unrelated to benzene exposure. The available information does not clearly support any of the pathways of leukemogenesis discussed here. Obviously an animal model of benzene leukemogenesis would be useful.

It has been suggested that benzene may act as a cocarcinogen, which would in part explain why only certain benzene-exposed individuals develop leukemia. An effect of benzene as a cocarcinogen or initiator might also explain cases in which initial hematotoxicity led to cessation of exposure, followed by a long delay before eventual development of acute leukemia.[6,49,83,98,116,150,162,166,193] Evidence on this subject is not firm.

A genetic predisposition has also been invoked to explain the response to benzene. Erf and Rhoads note possible hematotoxicity in brothers.[55] Aksoy and his colleagues present a number of cases of benzene hematotoxicity developing in families. These include two thalassemic brothers with pancytopenia, two cousins with pancytopenia, a nephew and paternal uncle with acute lymphoblastic and acute myeloblastic leukemia, a man with releukemia whose father had died of a possible acute leukemia, and two maternal cousins, one of whom had preleukemia and one an acute myelomonocytic leukemia. With one exception, a painter, these persons all worked in the shoe industry. Although these cases represent suggestive evidence of a genetic predisposition to benzene hematotoxicity, the degree to which family groups participate in the shoe industry has not been specified. If, as in many similar crafts, it is likely that most of the male family members will follow the same occupation, then the cases reported by Aksoy et al may not be greater than what is expected by chance. This would be true particularly if family groups were large and if the members tended to work in the same shops with the same benzene-containing adhesives. Accordingly, a genetic predisposition to benzene toxicity is not yet proven. Genetic factors also could be operative in determining the rate or extent to which benzene is metabolized to its presumed hematotoxic intermediate. Also of interest is an increased incidence of leukemia in a number of disorders that have in common an increased predisposition to chromosomal damage.[131]

The question of other individual host factors in susceptibility to benzene has been raised by a number of authors. Greenburg et al[82] suggested that obesity predisposes to hematotoxicity, presumably reflecting the solubility of benzene in fat. Although several investigators suggest that younger individuals and females are more susceptible to benzene toxicity,[53,96,118] this has not been confirmed in other studies. There is also conflicting evidence as to whether persons with beta-thalassemia minor, an inherited disorder of hemoglobin synthesis, may be at increased risk for benzene hematotoxicity.[4,65,70,157] If greater susceptibility is confirmed, this might indicate that more rapidly proliferating bone marrow, which is part of the thalassemia syndromes, increases the risk of hematopoietic cell damage due to benzene. Environmental factors, such as high ambient temperatures,[108] may also affect benzene toxicity. In addition, the ingestion or inhalation of food, drugs, or chemicals might modify the metabolism of benzene and hence its toxicity. At present the information is too meager for clear identification of individual host factors in development of pancytopenia or leukemia resulting from benzene exposure. Thus, it is unclear whether the marked variability in individual benzene hematotoxicity observed in occupational settings primarily reflects variations in benzene dose or operation of unknown host factors.

In summary, the principal mechanisms by which benzene could act as a leukemogen are considered to be 1) overt pancytopenia, 2) inapparent damage to bone marrow, 3) cocarcinogenic action, 4) response based on genetic predisposition, and 5) operation of coincident host factors.

OTHER BENZENE-ASSOCIATED DISORDERS

In addition to pancytopenia and acute myelogenous leukemia, which are the primary forms of toxicity associated with benzene exposure, various other hematological disorders also have been associated with benzene exposure, including some of the leukemia variants discussed earlier. We now consider some of the evidence relating benzene exposures to acute and chronic lymphatic leukemia, other lymphoproliferative disorders including Hodgkin's disease and multiple myeloma, chronic myelogenous leukemia, acute monocytic leukemia, myelofibrosis and myeloid metaplasia, thrombocythemia, paroxysmal nocturnal hemoglobinuria, and various disorders involving organ systems other than hematopoietic tissue.

Acute lymphoblastic leukemia is the usual form of childhood leukemia but is relatively rare in adults, in whom acute myelogenous leukemia is more common. It is sometimes difficult to distinguish acute lymphoblastic leukemia from other forms of acute leukemia by standard morphological techniques. Aksoy et al noted four cases of acute lymphoblastic leukemia in a group of 34 leukemic individuals.[10] The control population consisted of 50 leukemic individuals with no history of benzene exposure, of whom 26 had acute lymphoblastic leukemia. Goguel et al noted 2 cases of acute lymphoblastic leukemia in 50 cases of leukemia in benzene-exposed individuals.[77] Individual case reports of acute lymphoblastic leukemia associated with benzene exposure are also reported.[50,90]

More substantial evidence is available concerning an a ssociation of benzene exposure with chronic lymphatic leukemia. As described in detail earlier, McMichael et al[129] noted an association of chronic lymphatic leukemia with solvent exposure in a study of the cause of death of more than 6000 men employed in the rubber industry. (They observed no increase in acute leukemia.) In addition to a number of other individual case reports[34,37,54,69,102,142] and a collection of three cases by Tareef et al in the Soviet Union,[175] Girard and his colleagues in France have noted a statistically significant increase in history of benzene exposure in patients with chronic lymphatic leukemia as compared to control subjects without this disease.[72-75] In contrast to these findings, however, Aksoy et al[10] in Turkey and Vigliani et al[189,193] in Italy have not reported any cases of chronic lymphatic leukemia in their relatively large series. This apparent discrepancy in the observations of the French investigators and those in Italy and Turkey is puzzling. Chronic lymphatic leukemia is a disease of the elderly. Because substantial occupational exposure to benzene may have been discontinued in France at an earlier time period, cases of chronic lymphatic leukemia may eventually appear in the Italian and Turkish groups having more recent benzene exposure. Another possible explanation is that the risk of developing chronic lymphatic leukemia due to benzene exposure is modified by the presence of other solvents, which may have differed in France, Italy, and Turkey.

The evidence of a relationship between benzene exposure and chronic lymphatic leukemia is suggestive but not conclusive.

Lymphosarcoma,[35,40] Hodgkin's disease,[8,118] reticulum cell sarcoma,[141] and multiple myeloma[175,180] also are reported in association with benzene exposure. In none of these situations have there been enough case reports to imply other than a chance relation to benzene. Similarly, acute monocytic leukemia of the Schilling type is rarely related to benzene exposure.

Various myeloproliferative disorders are reported in association with benzene. A recent review tabulated 27 cases of chronic myelogenous leukemia.[78] Thirteen were from a series by Goguel et al[77] evaluating leukemia observed in benzene-exposed individuals from 1950 to 1965 in the Paris area. The authors note no clinical differences from cases of chronic myelogenous leukemia observed in the absence of benzene exposure. Many of the other case reports are also from France.[36,114] In addition, Tareef et al in the Soviet Union reported five cases of chronic myelogenous leukemia associated with benzene exposure.[175] One of these individuals eventually developed acute myeloblastic leukemia, a not infrequent outcome of chronic myelogenous leukemia.

Myelofibrosis and myeloid metaplasia is a relatively rare myeloproliferative disorder in which fibrosis of the bone marrow is accompanied by extramedullary hematopoiesis, particularly in the spleen and liver. An increased incidence of this disorder in atom bomb survivors is reported. Several case reports associate this disorder with benzene exposure.[64,125,144,196] Most recently, Aksoy et al[12] described a 35-year-old shoeworker with evidence of myelofibrosis and myeloid metaplasia including a diagnostic splenic aspiration. Nine years earlier, the patient had been diagnosed as having a benzene-induced pancytopenia due to an 8-year occupational exposure. Although these findings are suggestive, there are too few case reports to document a causal relationship between benzene and myelofibrosis and myeloid metaplasia.

Aksoy et al[6] recently reported another myeloproliferative variant, thrombocythemia, as a transient phenomenon in a shoemaker whose benzene-associated pancytopenia evolved into a disorder characterized by a high platelet count (540,000 to 1,600,000 per mm^3) and then into acute leukemia. Polycythemia vera, another myeloproliferative disorder, apparently has not been described in association with benzene exposure.

Paroxysmal nocturnal hemoglobinuria is a hemolytic disorder noted infrequently in benzene-exposed individuals.[12,46] This is an extremely rare condition that has been linked to both aplastic anemia and acute myelogenous leukemia. The disease is paraneoplastic, being defined by a population of circulating red cells that are abnormally sensitive to the hemolytic effect of complement. It often occurs in the setting of an aplastic anemia, may evolve into acute myelogenous leukemia or particularly erythroleukemia, and is sometimes noted as a relatively transient phenomenon during cases of acute myelogenous leukemia. Accordingly, its observation in workers exposed to benzene is not unexpected.

In addition to hematopoietic tissue, several other organ systems are said to be affected by benzene. Effects on the central nervous system following acute exposure to benzene have been clearly documented in man. These have been reviewed by Gerarde[66] and by Browning,[39] the latter noting 13 fatal cases in Great Britain following inhalation of high levels of benzene in enclosed areas. Reported effects following acute exposure include headache, nausea, staggering gait, paralysis, convulsions, and eventual unconsciousness and death. Recent reports of fatalities have been presented by Tauber[176] and by Bass.[21] Giddiness and euphoria also have been noted. These acute effects are usually observed only at relatively high levels of benzene, well above concentrations believed to be responsible for hematological effects following chronic exposure. Eastern European investigators have suggested recently, however, that effects on the central nervous system, including changes recorded by electroencephalograph and alterations in cerebral circulation, may occur in occupational settings.[134,169] There are also unconfirmed suggestions in the literature that the human cardiovascular[39,135,179] and gastrointestinal systems[20,40] may be affected by benzene.

SUMMARY

Benzene exposure has been clearly demonstrated to produce hematotoxicity in man. The most commonly reported effect is a decrease in one or more of the formed elements of the blood. In more severe cases, this takes the form of pancytopenia, often with aplastic bone marrow. Evaluation of occupationally exposed groups reveals a wide spectrum of disease ranging from fatal aplastic anemia to individual cytopenias and, in some studies, qualitative abnormalities of blood cells in the presence of normal peripheral blood counts. The evidence that benzene is causally related to pancytopenia includes the observation that benzene is the common denominator in outbreaks of pancytopenia observed in many different occupational exposure settings throughout the world, that detection of pancytopenia in a work force is often temporally related to the use of benzene, and that similar effects are observed in animals treated with benzene.

The causal relationship of benzene exposure to leukemia is more controversial, particularly because an animal model of this effect has not been clearly demonstrated. Recent studies, however, have provided strong confirmatory evidence of a causal relationship, which now appears to be beyond reasonable doubt for acute myelogenous leukemia and its variants. The evidence includes the many individual case reports of benzene-induced pancytopenia that proceed to acute myelogenous leukemia and erythroleukemia, the accumulated case reports of leukemia associated with benzene exposures, and, most importantly, the epidemiological evidence indicating a greater risk for leukemia among benzene-exposed individuals.

It would appear that the evidence indicating an increased risk of leukemia on exposure to benzene for various periods of time and at various concentrations is overwhelming. Unfortunately, the data are not adequate for deriving a scientifically valid dose-response curve. Such a curve may be estimated on the basis of various assumptions; these assumptions, however, usually represent hypotheses that, although they may be valid, are not yet proven. Hence the estimation of a dose-response curve is not appropriate in this report, which deals with the currently available scientific knowledge of health effects, but is undertaken in the benzene risk assessment document.

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